THE INFLUENCE OF FECAL EXTRACTS ON GERMAN COCKROACH (*Blattella germanica* (L.)) TRAIL FOLLOWING BEHAVIOR, INSECTICIDE EFFICACY AND TRAP CATCH

BY

DINI MICHELE MILLER

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1998

To Edwina Y. Travis-Chin who skipped diapers and went straight to college tuition.

ACKNOWLEDGMENTS

I would like to express my most sincere gratitude to the members of my committee, Dr. James Nation, Dr. Richard Brenner and Dr. O. Norman Nesheim, for their many contributions to my research and dissertation. I particularly want to thank Drs. James Nation and John Toth for their help with the aqueous fecal extract.

I am greatly indebted to my advisor, Dr. Philip G. Koehler, who has been the single most influential figure in my adult life. Dr. Koehler is directly responsible for all of my accomplishments and I am deeply grateful for his gentle guidance and patient encouragement.

I wish to thank the students, faculty, and staff of the UF Department of Entomology and Nematology for their friendship and support. Additionally, I would like to thank the members of the USDA-ARS CMAVE, Household Insects Unit for their hospitality and expertise.

I wish to extend my gratitude to my family for their participation in what has proved to be the best six years of my life. I thank my mother, Diana, for loving me throughout my diaper period and my less than exemplary teenage years. Additionally, I thank my mother and stepfather, Shane Shea,

for encouraging me from afar and keeping me up to date with the other members of my family. I also thank my stepmother, Edwina Travis-Chin, for being my greatest benefactor and dearest friend. Finally, I wish to thank my father, Michael R. Travis, whose picture I keep right above my computer to remind me why I am here.

I extend my deepest eternal love to Tim McCoy, my colleague in cockroaches, my best friend, my technical advisor and soon, my husband. Truly, I have found the entomologist of my dreams.

TABLE OF CONTENTS

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

THE INFLUENCE OF FECAL EXTRACTS ON GERMAN COCKROACH (*Blattella germanica* (L.)) TRAIL FOLLOWING BEHAVIOR, INSECTICIDE EFFICACY AND TRAP CATCH

By

Dini Michele Miller

December 1998

Chair: Philip G. Koehler
Major Department: Entomology and Nematology

Methanol extracts of German cockroach feces have limited potential in the urban environment due to their unpleasant color and odor. Therefore, an aqueous extract of cockroach feces was developed. Cockroach fecal material was first extracted in methylene chloride and then with water. This aqueous extraction (colorless and low odor) proved to be just as active as the methanol extract in eliciting cockroach aggregation behavior. The aqueous extract was also shown to enhance cockroach mortality when combined with spray formulations of chlorpyrifos and boric acid.

The original methanol fecal extract was used to evaluate cockroach trail following behavior. In arena tests cockroach movements were recorded in relation to "trails" of either fecal extract or methanol (control). Movement data were recorded as xy coordinates. From these coordinates the

average perpendicular distance of the cockroach from the extract trail was calculated. The average distance of the cockroaches from the fecal extract trail was found to be significantly less than the distance from the control trail. A dose response bioassay determined that a 5.7% concentration of extract in methanol was necessary to elicit trail following in 50% of the cockroaches (TC_{50}).

The aqueous extract was similarly evaluated for trail following activity. In arena tests the extract successfully elicited trail following behavior in adult male cockroaches. Additionally, trap catch in cockroach monitoring stations was significantly enhanced when trails of aqueous extract were applied between cockroach harborages and sticky traps.

The amine-alcohol, 1-dimethylamino-2-methyl-2-propanol (DMAMP), was previously identified as the most attractive component in German cockroach fecal material (aggregation pheromone). We evaluated DMAMP for eliciting trail following behavior. However, the cockroaches did not respond to DMAMP in the bioassay. When the methanol fecal extract was analyzed using gas chromatography in full scan electron impact, selected ion monitoring and chemical ionization modes, DMAMP was not detected. If present, DMAMP had a concentration in the extract of <50 ppm.

Although, cockroaches may detect pheromones at very low concentrations, DMAMP had failed to elicit trail following behavior. These results indicate that

"aggregation pheromone" is not responsible for trail following behavior and that another "trail pheromone" is present in cockroach feces.

CHAPTER 1
INTRODUCTION

The German cockroach is one of the most prolific and undesirable domiciliary pests in North America (Cornwell 1968, Benson and Zungoli 1997). It is most prevalent around homes, food processing plants and restaurants where warmth, food, and moisture are readily available (Cornwell 1968). Because of their strict moisture requirements, German cockroach infestations are almost always located in kitchen and bathroom areas (Cornwell 1968). German cockroach presence in kitchens and food handling areas is not only a nuisance but problematic because of the health concerns associated with food contamination. German cockroaches carry a number of pathogenic organisms on their bodies. These same organisms are also deposited with their feces (Morrell 1911, Jettmar 1935, Mackerras and Mackerras 1948, Cardone and Gauthier 1979, Strek et al. 1979, Alcamo and Frishman 1980). In infested situations cockroach feces accumulate in great quantities. It then becomes almost impossible to keep food products free of this material.

Although the possibility of food contamination can be very significant, allergies are the most important health issue associated with cockroach frass. German cockroaches

produce a tremendous amount of fecal material which contains allergens (Brenner et al. 1991). Allergens in cockroach feces and cast skins are very dry and easily become airborne. The allergenic particles are then inhaled by residents in infested structures (Koehler et al. 1990). In severely infested homes sensitivity to cockroach allergens may be as high as 79% in asthmatic children (Schulaner 1970). Chapman et al. (1992) stated that cockroach allergens in urban environments are a major risk factor for acute asthma attacks.

Although cockroach feces can be a problem in human structures, fecal material is an essential part of the German cockroach environment. Fecal material deposited in cockroach harborages is an important food source for young instars. First instar German cockroaches have great fidelity to shelters and extremely limited foraging ranges (Ross et al. 1984, Cloarec and Rivault 1991). Therefore, they seldom seek sources of food outside the harborage. Kopanic and Schal (1997) demonstrated that first instar German cockroaches will readily eat adult feces. This behavior increases their survivorship in the absence of other food sources. Additionally, first instar cockroaches will ingest adult feces even if alternative food sources are available, indicating that adult fecal material is an important source of nutrition for small nymphs (Kopanic and Schal 1997).

Fecal pellets are also used by German cockroaches as chemical signals. Numerous studies have documented the pheromonal properties of German cockroach fecal material. In fact, German cockroach aggregation behavior is mediated by volatile and nonvolatile chemicals contained in their feces (Ishii 1970, Bell et al. 1972, Burk and Bell 1973, Wileyto et al. 1984, Fuchs et al. 1985, Sakuma and Fukami 1993). These chemicals function first as attractants and then arrestants which halt cockroach movements once they make contact with the pheromone source (Sakuma and Fukami 1990). This behavior causes cockroaches to gather together in groups where their feces are prevalent. German cockroach fecal material tends to collect in harborages and areas where the temperature and humidity are optimal for cockroach survival (Denzer et al. 1988). The presence of the fecal material chemically advertises these prime habitat locations to cockroaches that are new to the environment or that are returning to particular harborages after foraging.

In addition to facilitating aggregation and harborage identification, German cockroach feces have long been suspected of providing navigational cues, specifically, guiding cockroaches to and from their harborages during foraging (Kitamura et al. 1974). Studies have shown that extracts of German cockroach fecal material influence chemo- and anemo-taxes as well as the direction of walking behavior in adult males (Sukuma and Fukami 1985, Wendler and Vlatten

1993). However, extracts of German cockroach fecal material have not been evaluated for use as a navigational tool. The following studies were designed to address the use of fecal material by German cockroaches for directional orientation or trail following behavior. One of our first objectives was to develop a simple extraction process for cockroach fecal material that would maintain the attractant activity yet be suitable for use in the urban environment. The next step was to determine if German cockroaches would actually use their fecal material for directional orientation. The ability to exploit this trail following behavior was then examined by creating artificial trails between cockroach harborages and sticky traps used for cockroach monitoring. Finally, gas chromatography and mass spectrophotometer was used to determine the role of a volatile aggregation pheromone molecule, 1-dimethylamino-2-methyl-2-propanol (DMAMP), in German cockroach trail following behavior.

German cockroach feces are highly undesirable in human living space. However, an understanding of how German cockroaches make use of this material can be very important in the manipulation of their behavior and their environment. As chemical control methods become less welcome inside human structures, control methods that depend less on toxicants and more on natural cockroach behavior become increasingly more valuable.

CHAPTER 2
INFLUENCE OF FECAL MATERIAL ON GERMAN COCKROACH BEHAVIOR

Introduction

Despite all of our heroic efforts to manage, suppress, and eliminate German cockroaches from our living spaces, they still remain the most prevalent and annoying structural pest in the United States (Rust et al. 1996). Large populations of these pests frequently occur in situations where sanitation is low and environmental conditions are optimal for their proliferation (Gupta et al. 1975). Unfortunately, homes, restaurants and food processing plants are some of the most preferred habitats of German cockroaches. These locations often provide incipient populations with numerous sources of warmth, food, water and harborage. Yet, it is in these locations that their presence is the least desirable (Cornwell 1968).

Until recently, efforts to suppress cockroach populations in the urban environment relied heavily on repeated applications of synthetic pesticides. At first, residual spray and dust formulations were very effective. Yet, our extensive use of these inexpensive, easy application chemicals was not without consequences. Repeated applications of the same or similar toxicants over

time led to the development of pesticide resistance in German cockroaches. This allowed genetically tolerant cockroaches to survive pesticide application and quickly reestablished populations in chemically treated locations. Even more discouraging was the fact that these new populations of cockroaches were no longer susceptible to insecticide treatment.

In addition to the frustrations caused by pesticide resistance, increased concern among consumers and regulatory officials about indoor chemical exposure severely reduced the popularity of broad application residual insecticides. Consequently, pest control operators found themselves in a very difficult position. On the one hand, consumers and regulators were demanding that dangerous chemicals be eliminated from human living spaces. Yet at the same time, these individuals were demanding that the urban environment be free of pests as well.

These concerns about pesticide resistance in cockroaches and chemical exposure did not go unheeded by the pest control products industry. Many manufacturers began to move away from spray and dust formulation insecticides to new products that were environmentally friendly and more specifically targeted at cockroach pests. Manufacturers began to study cockroach ecology and behavior in order to design products that could take advantage of the cockroach's natural activities. Crack and crevice treatments, baits and

monitoring traps are all part of the new generation of products that depend less on toxicants and more on natural cockroach behavior.

One of the key behavioral components that many of these new control products exploit is German cockroach aggregation behavior. For example, crack and crevice treatments are directed at cockroaches in the harborages where they aggregate. Cockroach baiting, an old control technique, has been completely renovated with new toxicant chemistries and novel delivery systems that are directed at aggregating cockroaches. In fact several bait formulations allow for numerous small applications to be injected or sprayed right into harborages that house large portions of the cockroach population. Even monitoring traps have been redesigned to include pheromone attractants that exploit cockroach aggregation behavior. These natural cockroach odors lure cockroaches into the traps in search of shelter and conspecifics.

Although German cockroach aggregation in domestic harborages is very well documented, the mechanisms responsible for this behavior were elucidated only after years of research. In this review we will examine many of the studies that have investigated German cockroach aggregation behavior. The pheromonal activity of cockroach fecal pellets and the identification of the chemical components responsible for aggregation behavior will be

discussed. Additionally, we will examine those studies in which "aggregation pheromone" was evaluated for its influence on German cockroach movement behavior. These studies are of particular interest because they suggest the possibility of pheromone mediated trail following behavior in the German cockroach. Finally, we will look at evolutionary models for trail following behavior in social insects and examine how these models apply to German cockroach trail following behavior.

Aggregation Behavior

Ledoux (1945) was the first to discuss aggregation behavior in German cockroaches and suggested that chemical stimuli played a significant role in this behavior. Ishii and Kuwahara (1967) were able to show that the gregariousness in German cockroach nymphs depended on a chemical substance that was contained on the body surface and in the feces. Ishii (1970) later found that this chemical substance initiated German cockroach aggregation behavior in all life stages. Several researchers have determined that German cockroach aggregation is a two part process in which chemicals in the cockroach frass act first as attractants then as arrestants once tactile contact is made with the pheromone source (Ishii 1970, Bell et al. 1972, Burk and Bell 1973, Wileyto et al. 1984, Fuchs et al. 1985, Sakuma and Fukami 1993). In experiments where German cockroach nymphs were given a choice of filter papers to

rest on, one clean versus one that had been stored in a cockroach rearing container for several days, the nymphs tended to aggregate on the filter paper contaminated with cockroach feces. This preference for the "seasoned" filter paper was shown to disappear if the cockroach antennae were removed. Thus, it was concluded that aggregation behavior was mediated by substances contained in the cockroach feces and perceived through the antennae (Ishii 1970).

Although the chemical(s) responsible for the aggregation behavior were not identified at that time (1970), the site of active chemical secretion was determined through a series of biological assays. Adult male cockroaches were cut into portions consisting of the head, legs, wings, thorax and abdomen. These dissected sections were extracted in ether and the extracts were applied to filter paper. Each extract was assayed on filter paper for aggregation behavior as described above. The results of the aggregation bioassay indicated that the active portion was mainly located in the cockroach abdomen. Further investigation determined that cells in the cockroach rectal pads were responsible for the secretion of "aggregation pheromone". Once produced, the aggregation pheromone is secreted into the lumen and then passed with the fecal wastes (Ishii 1970).

Although the aggregation pheromone itself was not identified, the Ishii (1970) study initiated a great deal of

research to determine the role of "aggregation pheromone" in German cockroach ecology. One of the first and most interesting questions investigated had to do with the potential evolutionary advantage of aggregation behavior in a domestic cockroach species. In theory, aggregation should occur when individuals attain greater fitness by remaining in the group than they would if they were alone (Alexander 1974). Several possibilities for this behavior have been suggested. Pulliam (1973) stated that the aggregation of many individuals helps each to avoid predation. The scattering of the group when threatened confuses predators thus benefitting all of the group members. Domestic cockroaches have very few natural enemies other than humans and possibly domestic rodents, but the impact of these "predators" on German cockroach populations is difficult to determine (Cornwell 1968).

Another suggested possibility for aggregation behavior is the amelioration of the local environment by the group, thus promoting individual survival (Wileyto et al. 1984). Ishii and Kuwahara (1967) and Izutsu et al. (1970) provided a reasonable argument for environmental amelioration by demonstrating that cockroaches grow more rapidly in a group than when reared in isolation. Additionally, their age to reproductive maturity significantly decreased. However, the data also indicated that adult weight and juvenile survival were decreased with increased group size (Ishii and Kuwahara

(1967). So, aggregation and amelioration of the local environment did not necessarily promote individual survival.

A third explanation for cockroach aggregation would be to facilitate mating. Wileyto et al. (1984) demonstrated that adult males were most attracted to the fecal residues of virgin females. Yet, Ross and Tignor (1986) found that all classes of cockroaches except middle instars and gravid females were also significantly more attracted to the fecal material of nongravid females. Therefore, attraction to female feces does not appear to be specific to receptive males. Wileyto (1984) also found that nongravid females were no more attracted to adult male fecal pellets than those of gravid females or nymphs. So, although, the male attraction and aggregation on virgin female feces would serve to aid males in the location of mates, the theory of aggregation for the facilitation of mating does neither explains aggregation behavior in gravid females nor juvenile cockroaches.

A more reasonable explanation for German cockroach aggregation behavior may be the identification of "safe" or preferred harborages. Denzer et al. (1988) determined that aggregation behavior induced by pheromones contained in the fecal pellets was a main element in German cockroach spatial distribution. Although, all cracks that were wide enough to accommodate the cockroaches could be used as harborage, the most preferred harborage was marked with the fecal material

of the home group. These groups of German cockroaches displayed great loyalty to particular harborages and there was very little movement between harborages that housed different portions of the population. The presence of the fecal material advertises that the home harborage is safe or beneficial as demonstrated by the recognizable presence of conspecifics (Wileyto et al. 1986).

One advantage of aggregation in particular harborages is that the presence of numerous conspecifics helps to maintain a stable microhabitat. Large numbers of metabolizing cockroaches in a closed environment such as a crack or crevice would help to stabilize temperature and humidity levels as well as reduce air flow through the harborage. This clustering of bodies would be particularly beneficial to small nymphs which are at the greatest risk of dehydration until they are old enough to forage for free water. An additional benefit for cockroach nymphs in the harborage would be the presence of adult feces. Adult fecal material has been shown to be an important food source for first instars which do not venture far from the harborage (Kopanic and Schal 1997).

Although none of the above theories have been proven definitively, harborage identification appears to be the most probable evolutionary advantage of German cockroach aggregation behavior. However, most animal behaviors are more complex than can be explained by a single hypothesis

and it is likely that all of the above hypotheses play at least a partial role in German cockroach aggregation behavior.

In addition to the basic research conducted to determine the biological advantages to German cockroach aggregation behavior, many applied studies were conducted to determine how aggregation behavior might be exploited to improve cockroach control methods. Ebeling et al. (1966) demonstrated that German cockroaches avoid surfaces treated with spray formulation insecticides. It was speculated that the cockroaches in the field may also avoid repellent insecticides hereby severely reducing blatticide efficacy. In 1971, Bennett and Wright observed that the repellency of certain spray formulation constituents on wood panels was significantly reduced when the panels were exposed to cockroaches 24 hours prior to treatment. Rust and Reierson (1976) conducted laboratory tests to determine if an extract of cockroach fecal residues could enhance the efficacy of repellent blatticides. Six insecticides used for German cockroach control were tested with and without the addition of German cockroach fecal extract ("aggregation pheromone"). It was found that when the toxicants were combined with the fecal extract ("aggregation pheromone") cockroach mortality was significantly increased. The authors suggested that the addition of the fecal extract reduced repellency of the insecticide and increased the amount of time that the

cockroaches remained in contact with the toxicant. Thus, increasing the possibility of cockroaches to picking up a lethal dose (Rust and Reierson 1976). Similar results were achieved when Rust and Reierson (1977) tested insecticide enhancement in the field. An extract of German cockroach feces was added to chlorpyrifos (0.25%) and diazinon (0.5%) in field tests treating infested apartments. Cockroach mortality was significantly increased for both toxicants when the fecal extract was applied with the insecticide. Glaser (1980) also reported increased cockroach mortality when a fecal extract was added to a wettable powder formulation of fenitrothion.

Although the addition of German cockroach fecal extracts have been shown to significantly enhance many spray formulation insecticides, the typical methanol extracts have such a disagreeable odor and color that they have been unacceptable for broad application indoors (Rust and Reierson 1976). However, in 1998, Miller developed an aqueous extract of German cockroach fecal pellets that is colorless and low odor (Chapter 3). This formulation has also been shown to enhance spray formulation of chlorpyrifos and boric acid. The water based extract has the potential to be used indoors for the enhancement of current cockroach control strategies.

Spray formulations are not the only cockroach control method that have been improved with the addition of fecal

extract "aggregation pheromone". Extracts of German cockroach fecal material have also enhanced the attractiveness of bait stations and improve toxic bait performance in the presence of competing nontoxic food sources (Miller et al. 1996,1997). When a methanol extract of German cockroach fecal material (100 µl) was placed next to a food source inside a bait station, a significantly greater number of German cockroaches chose to harbor in the station that contained the fecal extract. Additionally, more food was consumed in the extract treated bait station because more cockroaches were located inside (Miller 1996). In arena tests where five nontoxic food sources surrounded a single toxic bait station, mortality was significantly increased when a fecal extract was paired with the toxic bait (Miller 1997).

Identification of Aggregation Pheromone

Attractant components. In 1990, Sakuma and Fukami isolated and identified the attractive pheromone components that were responsible for the initiation German cockroach aggregation behavior. These components were isolated from filter paper contaminated by cockroaches. Successive purification steps were evaluated for attractiveness to cockroaches in an olfactometer assay measuring positive cockroach chemo- and anemotaxis toward each component. Every component that demonstrated attractant activity in the bioassay was identified by GC-MS analysis. The attractant

components of the pheromone were found to be comprised of a suite of volatile amines. Some of the major attractants were identified as ammonia, methylamine, dimethylamine and triethylamine. However, one component was found to be 50 to 1000 times more active than all of the other amines. This component was identified as 1-dimethylamino-2-methyl-2-propanol or DMAMP (Sakuma and Fukami, 1990).

DMAMP and the other attractant amines are known to be highly volatile (Sakuma and Fukami, 1996), so volatile in fact, that we would expect the attractants to evaporate almost immediately after application onto a substrate. However, in the natural state within the fecal pellet, the attractive amines remain active for a year or longer (Rust et al. 1995). This slow release process of attractant amines and DMAMP within the fecal pellets has been found to be controlled by acid components also present in the cockroach fecal material (Ritter and Persoons 1975, Sakuma et al. 1996a). The acids are combined with the alkylamines and amine alcohols as salts in the natural state. These salts then allow the amine attractants to be released slowly over time. In the laboratory it was found that the recombination of the attractant amines with an acid was a prerequisite for the controlled release of the attractants from a substrate (Sakuma et al. 1996b).

Sakuma and Fukami (1996a) used ion chromatographic analysis to identify the acids present in German cockroach

fecal material. It was found that hydrochloric acid (HCl) was the major acid constituent present in cockroach feces. HCl accounted for 81% of the total equivalent of anions and acids in the aqueous fraction of a crude cockroach fecal extract.

It is interesting to note that humidity was found to negatively affect the release of attractant amines from the hygroscopic salts (chlorides). Triethylamine hydrochloride was bioassayed on a disc dispenser at various relative humidities and it was found that attractant activity was significantly reduced at relative humidities greater than 50% (Sakuma et al. 1996a). This decrease in attractiveness is attributed to the phase transition from the salt solid to solution which reduces the amine vapor pressure (Pio and Harrison, 1987).

Arrestant components. In 1993(a), Sakuma and Fukami isolated and identified the arrestant components of German cockroach aggregation pheromone. The arrestant components were extracted along with the attractants described above from frass-contaminated filter paper. The arrestants were separated from the attractants with *n*-butanol and purified by open column chromatography. HPLC was then used to isolate two major arrestant components (Sakuma and Fukami 1993b). They were identified as novel steroid glycosides, and named blattellastanoside A and blattellastanoside B (Sukuma and Fukami 1993a). Both arrestants were tested for

activity in choice chamber assays where they were found to function as contact chemicals. An assay of antenn- and palpectomized cockroaches indicated that these contact pheromones were perceived with both the antennae and the maxillary palpi through contact chemoreception (Sukuma and Fukami 1993b). The blattellastanoside A was reported to be 70 times more biologically active than B as an arrestant compound (Mori 1996).

The structural identification of the blattellastanoside A and B were confirmed through synthesis by Mori (1996). It was also demonstrated that these two steroid glycosides had next to no volatility confirming their description as contact pheromones. In attempting to synthesize analogs of blattellastanoside A it was found that the steroidal side-chain and the D-glucose moiety of the pheromone were essential to the bioactivity of the pheromone. However, a fluorinated analog of the blattellastanoside A was found to be more active than the natural compound. This was unusual because it is generally accepted that the natural pheromone is the more readily perceived by the insect (Mori 1996).

Combined Function of Phermone Components. Aggregation behavior in German cockroaches is currently thought to be a function of both the attractant and arrestant pheromone components acting in concert to bring large numbers of cockroaches together in one location. In the natural environment the volatile amine attractants evaporate slowly

from German cockroach fecal pellets. These attractants are thought to function by inducing orientation behavior such as chemo- and anemotaxis (Sakuma and Fukami 1985) that guides cockroaches toward the odor source. Next the nonvolatile, arrestant components of the aggregation pheromone act as chemical signals to produce a reverse orthokinesis response (Kennedy 1978) which halts cockroach movement once contact has been made with the pheromone source (Sakuma and Fukami 1993b). In this way the combined attractant and arrestant components of German cockroach aggregation pheromone focus the local search capability. As stated previously, this behavior may have the adaptive advantage of helping the cockroaches recognize suitable harborage locations.

Aggregation Pheromone and Cockroach Movement

Several studies have investigated the influence of pheromones on peridomestic cockroach directional orientation and movement. Rust et al. (1975) and Tobin (1981) looked at pheromone stimulated locomotory and directional orientation responses in American cockroaches to sex pheromones secreted by females. Both researchers found that adult males increased locomotion and positive chemo-orientation in the direction of a female sex pheromone. Bell et al. (1973) investigated the influence of fecal material ("aggregation pheromone") on American cockroach directional orientation. Using a T-maze, Bell et al. (1973) found that American cockroaches preferred to walk on the portion of the maze

that was contaminated with their fecal material. Further evaluation revealed that the tropochemotactic orientation of the cockroaches to the maze was dependent on olfactory perception of the "aggregation pheromone" by the antennal receptors (Bell et al. 1973).

Pheromone mediated movement and directional orientation have also been investigated in German cockroaches. German cockroach movement in response to volatile extracts of their fecal material has been determined in assays using a linear track olfactometer (Sakuma and Fukami 1985, 1990). In studies observing chemo- and anemotaxis in German cockroach nymphs, strong positive taxis to frass contaminated shelters was observed (Sakuma and Fukami 1985). Additionally, the positive chemo- and anemotaxis were found to increase consistently with increased doses of German cockroach fecal extract "aggregation pheromone" (Sakuma and Fukami 1990). When one antenna was removed, the cockroach nymphs still displayed positive chemo- and anemotaxis. However, bilaterally antennectomized nymphs exhibited neither chemo- nor anemotaxis. These experiments confirmed the Ishii (1970) study which indicated that volatile chemicals present in the fecal extracts were detected via the cockroach antennae (Sakuma and Fukami 1985).

Like the American cockroaches (Bell et al. 1973), German cockroaches have also been tested in a T-maze to determine the role of fecal material on their directional

orientation (Kitamura et al. 1974). In tests where methanol extracts of German cockroach feces were applied to the left or right branch of the maze it was found that the German cockroaches preferred to walk along the side where the fecal extract was applied. The Kitamura et al. (1974) study indicated that some of the fecal components that elicited the directional movement were not volatile but were perceived by contact or short range chemoreception through the antennal receptors. They suggested that it was these contact chemicals that German cockroaches used to navigate to and from their harborage locations when foraging (Kitamura 1974).

The suggestion that German cockroaches use their fecal material for the purpose of navigation poses an interesting question about German cockroach search behavior. Do German cockroaches use chemicals in their fecal material as a possible trail pheromone?

Bell (1991) stated that movement along particular paths reflect the attention that animals (insects) give to directional cues in their environment. If cockroach fecal pellets are considered to be chemical cues as indicated by Kitamura (1974) then the study of feces distribution in arena tests performed by Stejskal (1997) clearly indicates that cockroach fecal material aids in their navigation between the shelter and resources in their environment. Stejskal (1997) recorded the pattern of cockroach feces

deposition in a glass arena during the colonization of new refuges. It was determined that the largest number of fecal pellets were deposited in and around the harborage. The second largest number was recorded around the edges of the arena where the cockroaches passed repeatedly on foraging bouts between the harborage and sources of food and water. Because cockroaches are thigmotropic, preferring to follow edges of walls and floors, it stands to reason that fecal pellets would tend to accumulate in these frequently traversed areas. The cockroaches can then use these fecal pellets as odor trails for navigation to and from the harborage in the same way that ants, termites and the gregarious eastern tent caterpillars (Fitzgerald and Edgerly 1979) use chemical trails to navigate to and from their nests.

Perhaps the best study documenting German cockroach trail following behavior was conducted by Wendler and Vlatten (1993) whose intention was to evaluate the influence of "aggregation pheromone" on German cockroach walking behavior. In this study, adult male German cockroaches were tested on a locomotion compensator. The cockroaches had constant antennal contact with a source of fecal extract ("aggregation pheromone") on a strip of filter paper. It was found that when the cockroaches made tactile contact with the fecal extract, their previously non-directed walking pattern was immediately changed and oriented in such

a way as to maintain contact with the treated filter paper. The cockroaches did not orient or move toward control filter papers indicating that orientation behavior was significantly influenced by the fecal extract (Wendler and Vlatten 1993).

The most important aspect of this study in relation to trail following behavior lies in the interpretation of the cockroach movement data. Wendler and Vlatten (1993) found that the average walking speed and the number of stops made by the cockroaches were reduced as antennal contact ("overlap") with the fecal extract treated filter paper increased. However, the length of the time that the cockroaches remained standing during the test period increased from 20% when the filter paper was 6 mm away to 74% when the cockroach had 5 mm of antennal (tactile) overlap with the extract treated filter paper. Wendler and Vlatten (1993) interpreted this increase in standing time as the precursor in cockroach aggregation behavior. They stated that in field conditions the attainment of full contact (= maximal overlap (not defined)) would lead to a full cessation of cockroach movement. However, a full cessation of cockroach movement was not observed in their laboratory study. Additionally, the authors conceded that the pheromone stimulated locomotory behavior witnessed in their study is also seen in insects displaying no aggregation behavior.

I suggest that the observed orientation behavior consisting of fewer, slightly longer stops and reduced speed is more indicative of trail following behavior than aggregation. The cockroaches move consistently in the direction of the fecal extract in order to remain in constant contact with the pheromone. Additionally, Wendler and Vlatten (1993) state that it is the contact chemoreceptors on the cockroach antennae that are responsible for driving this locomotory behavior. This observation of directed movement mediated by short-range chemoreception of a pheromone is consistent with the trailing following behavior that is observed in other trailing insects such as ants, termites, and the eastern tent caterpillar.

Cockroach Trail Following: An Evolutionary Model

The literature indicates that there are actually two different behaviors associated with German cockroach fecal material. First, there is the aggregation behavior. Aggregation behavior is initiated by the detection of volatile attractant chemicals via the antennae. The cockroach moves toward the attractant source and then stops moving once contact is made with the pheromone source. Trail following behavior is initiated and maintained by constant tactile contact with nonvolatile chemicals in the fecal material. The contact stimulates continuous forward movement in the direction of the pheromone source.

This dual pheromonal function of the German cockroach fecal material is not unique. Wilson (1961) found this same pheromonal dual function in ants. Wilson (1961) demonstrated that secretions from the Dufour's gland among the worker's of the fire ant, *Solenopsis saevissima* (Fr. Smith) functioned both as trail pheromones and aggregation pheromones which caused the ants to aggregate together in large groups.

Unlike cockroaches, trail following behavior in ants and other social insects has been well documented. Yet, an evolutionary model suggested by Maschwitz (1975) for trail following behavior in social insects is also well suited for German cockroaches because of their loyalty to harborages and foraging areas contaminated with their fecal material (Denzer et al. 1988). Maschwitz (1975) suggested that trail following insects (ants) would defecate outside the nest in particular places that were frequented by conspecifics. The characteristic odor was then an ideal predisposition for signaling highly frequented places such as shelters and food sources. Subsequently, odorous chemicals in the fecal material took on an orientation function. Holldobler (1984) went further to argue that the use of the hindgut contents as a trail pheromone in formicine ant species evolved from a gradual ritualization of the defecation process.

Although ritualized defecation may explain the rise of trail following behavior in ants and even termites, there is

no evidence to suggest that cockroaches defecate in any recognizable pattern. Instead the more passive predisposition for pheromone signaling described by Maschwitz (1975) is most likely responsible for German cockroach trail following behavior. In established populations, trails would develop in areas that were frequented regularly by numerous cockroaches moving back and forth between their harborages and sources of food and water. Because these chemical trails would be constantly reinforced, new additions to the population either by birth or immigration would not have to search randomly for resources but would simply follow along the paths of their conspecifics. In this way the fitness of all of the individual cockroaches would be increased by reducing the amount of energy expended to locate resources in their environment.

CHAPTER 3
AN AQUEOUS EXTRACT OF GERMAN COCKROACH FECAL PELLETS TO ENHANCE THE EFFICACY OF SPRAY FORMULATION INSECTICIDES

Introduction

Insect pheromones have been used extensively to trap and manipulate agricultural pests, yet they have seen very limited use in the urban environment. With little exception, urban pheromone use has been restricted to monitoring stored products pests (Campion 1984). Structural urban pests such as ants and termites are completely dependent on pheromones, but we have been unable to exploit this dependence due to their predominantly subterranean existence. The German cockroach is a major structural pest that provides pest control operators with the dubious advantage of being readily accessible, i.e., living above ground and in close association with humans. This choice of habitat makes the German cockroach an excellent target for manipulation using pheromones, particularly their aggregation pheromone.

Cockroach aggregation pheromone functions primarily as a cockroach attractant and secondarily as an arrestant (Burk and Bell 1972, Sakuma and Fukami 1990, 1993), thus attracting cockroaches to a particular area and arresting

their movements once tactile contact is made with the pheromone source (Sakuma and Fukami 1993). Aggregation pheromone is attractive to both sexes of the German cockroach and all nymphal life stages (Ishii and Kuwahara 1968). Additionally, the pheromone is not strictly species specific but is attractive to another Blattellid species, *Blattella asahinai* (Appel 1995).

Aggregation pheromone attractant activity was first documented in German cockroach fecal material by Ishii and Kuwahara (1967). The active component was not yet identified but histological studies indicated that the active component was secreted from the rectal pad cells into the rectal lumen of the cockroach and then passed in the feces (Ishii 1970).

The documentation of the attractant activity of German cockroach fecal material prompted several studies by Rust and Reierson (1976, 1977) to evaluate the application potential of aggregation pheromone. A methanol extraction of German cockroach fecal material was added to repellent insecticides to see if cockroach mortality could be increased. These studies proved that the addition of fecal extract significantly enhanced the performance of several repellant toxicants. Unfortunately, the authors noted that the extract had both a disagreeable color and odor making indoor applications undesirable (Rust and Reierson 1976). As a result of the Rust and Reierson (1976) study,

aggregation pheromone research in terms of practical application was not pursued for many years.

In 1990 Sakuma and Fukami isolated, identified, and synthesized the most attractive component of German cockroach aggregation pheromone. Identified as 1-dimethyl-amino-2-methyl-2-propanol, it was reported to be soluble in polar solvents, water and methanol. The most important characteristics of this molecule, in terms of application potential, was its description as being both colorless and odorless (Sakuma and Fukami 1990).

The solubility of German cockroach aggregation pheromone plus its lack of color and odor reopened the possibility of the pheromone being formulated for use indoors. The focus of this study was to develop a simple extraction process that would capture the attractant components present in German cockroach fecal pellets yet remove the disagreeable color and odor. The ability of this extract to enhance spray formulation insecticides was then evaluated and compared with the methanol extract described by Rust and Reierson (1976).

Materials and Methods

Aqueous extract preparation. Orlando strain German cockroach feces were collected from the bottom of mass rearing containers. Feces (20 g) were separated from extraneous debris by sieving the fecal pellets with a No. 20 (0.84 mm) sieve. Sieved feces (20 g) were added to 40 g of

methylene chloride for a 1:2 wt:wt feces:methylene chloride mixture. The fecal preparation was held in a 125 ml Erlenmeyer flask sealed with parafilm (American Can Company, Greenwich, CT.) for 24 h at room temperature (24°C). The supernatant was vacuum filtered through a Whatman No. 1 filter paper in a Buechner funnel. The filtrate was transferred into a 500 ml separatory funnel and shaken with an equal volume of water. The methylene chloride/water mixture was allowed to separate overnight. The clear aqueous phase was then removed with a glass pipette and stored in a capped amber wide-mouth glass bottle (250 ml; Fisher Scientific Pittsburgh, PA) at 0°C.

Methanol extract preparation. Feces from the Orlando strain of German cockroaches were collected from the bottom of mass rearing containers. Twenty grams of feces were separated from debris by sieving with a No. 20 (0.84 mm) sieve. Sieved feces (20 g) were added to 40 grams of methanol for a 1:2, wt:wt, feces: methanol mixture. The feces mixture was held in 125-ml Erlenmeyer flask sealed with parafilm (American Can Company, Greenwich, CT.) for 24 h at room temperature (24°C). The supernatant was vacuum filtered through a Whatman No.1 filter paper in a Buechner funnel and the extract was refrigerated in a capped scintillation vial (20 ml) at 0°C.

Cockroaches. First to third instar German cockroaches (Orlando strain) were obtained from the University of

Florida Urban Entomology Laboratory in Gainesville, Florida. Cockroach rearing was conducted under the regimen outlined by Koehler and Patterson (1986) at 26° C, 55% RH and a photoperiod of 12:12 (L:D) h.

Toxicants. Two spray formulation insecticides were chosen for testing based on their being currently labeled for indoor cockroach control and their similarity to products tested in the Rust and Reierson (1976) study. Dursban Pro, (22.5% A.I.; DowAgrosciences, Indianapolis, IN) an emulsifiable concentrate formulation of chlorpyrifos, is a fast acting toxicant and comparable to the Dursban 2E formulation used by Rust and Reierson (1976). Whitmire PT240, (Whitmire Research Laboratories, Inc., St. Louis, MO) is an aerosol formulation of boric acid. The PT240 is non-repellent and a relatively slow acting (Klotz and Moss 1996, Strong et al. 1993) and is analogous to the technical boric acid used by Rust and Reierson (1976).

Insecticide enhancement bioassay. The assay protocol for insecticide enhancement was similar to the "bowl assay procedure" described by Rust and Reierson (1976). Rectangular strips of Whatman No. 1 filter paper (3 x 7 cm) were treated with one of the following insecticide combinations: chlorpyrifos alone (0.1 ml of 0.26% concentration v:v, label rate formulation; applied with a micropippette); chlorpyrifos (0.1 ml) combined with the aqueous fecal extract (0.25 ml); chlorpyrifos (0.1 ml)

combined with the methanol fecal extract (0.25 ml); boric acid alone (applied at label rate of 1 sec/linear ft); boric acid (1 sec/linear ft) combined with the aqueous extract (0.25 ml; applied with a micropipette); boric acid (1 sec/linear ft) combined with the methanol extract (0.25 ml); aqueous extract alone (0.25 ml); water (0.25 ml). All treated filter papers were dried for 24 h before cockroach exposure.

For each test 20 early instar (1st-3rd) cockroaches were transferred without anesthesia to glass utility jars (4 liter). Three strips of filter paper were folded into a Z-shape and set on edge in a rectangular pattern at the bottom of the jars. Each jar contained 2 strips treated with water (controls) and one treated with a toxicant, an extract, a toxicant-extract combination or water only. Each treatment had 8 replications and all assays were begun after 9:00 pm with a photoperiod of 12:12, L:D (scotophase beginning at 10:30 pm). The number of dead nymphs was recorded at 15 and 24 h after treatment then the strips were removed, and the cockroaches provided with food and water. Subsequent mortality was recorded at 72 h.

Statistical analysis. Mean cockroach mortality was analyzed using the analysis of variance (ANOVA). Significant differences ($P \leq 0.05$) were separated using Fisher's test of Least Significant Difference (SAS Institute 1988).

Results

Aqueous extract. A solvent suitable for the extraction of the attractant components in the fecal material was preliminarily investigated. Sakuma and Fukami (1990) stated that only polar solvents extracted the attractant completely. Although methanol was an excellent solvent it produced an extract that was dark in color, contained large amounts of fatty acids (McFarlane, 1984; McFarlane and Alli 1986; Ritter and Persoons, 1975) and had an unpleasant smell (Sakuma and Fukami 1990). Therefore, a two step process was developed in which the fecal material was first extracted in one polar solvent, methylene chloride, and then further extracted with water. Water proved ideal for the final extraction because it was convenient to handle and nontoxic.

The final products of both the aqueous and methanol extractions are compared with tap water in Figure 3-1. The aqueous extract was quite clear and difficult to distinguish from the water except by smell. Although the aqueous extract lost the "roachy" odor during the extraction process it still retained an odor similar to that of stale water.

Insecticide enhancement bioassay. Mean nymphal mortality in trials where chlorpyrifos was combined with the aqueous extract was significantly higher at 15, 24 and 72 h (79% at 72 h) ($F = 18.3$, df = 4, $P < 0.05$) than either the chlorpyrifos alone (46% at 72 h) or the chlorpyrifos

combined with the methanol extract (28% at 72 H) (Table 3-1). Interestingly, where the methanol extract was combined with chlorpyrifos mortality was not significantly different from the chlorpyrifos alone.

Both the aqueous and methanol extracts produced significantly greater mortality at 15 h after treatment in trials where they were combined with the boric acid (Table 3-2). At 24 h, the aqueous extract continued to enhance mortality (42%) compared with boric acid (25%). After the treated surfaces were removed (72 h), mortality was not significantly different for either of the extracts. Yet, in trials where the methanol extract was combined with the boric acid mortality was significantly greater than those with the boric acid alone.

Discussion

Ebeling et al. (1967) was the first to add chloroform extracts of cockroach fecal material to repellent blatticides, but they were unable to increase the rate of mortality in adult male German cockroaches in choice boxes (Ebeling et al. 1967). However, Bennett and Wright (1971) were successful in demonstrating that the repellency of certain spray constituents could be reduced or eliminated by the presence of cockroach fecal deposits or "roachy odor." Bennet and Wright (1971) went on to suggest that the "roachy odor" (aggregation pheromone) factor could be used to lure cockroaches into areas that had been treated with repellent

insecticides. When Rust and Reierson (1977) confirmed that a repellent toxicant could be enhanced by the presence of German cockroach aggregation pheromone (fecal extract) they noted that the increased effects were not found when aggregation pheromone was formulated with non-repellent toxicants like boric acid (Rust and Reierson 1977).

The ability of the aqueous extract to enhance the insecticidal activity of the chlorpyrifos in this study was similar to the results obtained with the methanol extract tested by Rust and Reierson (1977). Rust and Reierson (1977) found that chlorpyrifos 2E combined with fecal extract (methanol) produced significantly greater mortality after 72 h than chlorpyrifos 2E alone (91% versus 74.3%). In our study the chlorpyrifos/aqueous extract combination significantly enhanced mortality, killing 78.6% of the cockroaches in 72 h.

The poor performance of the methanol extract in the chlorpyrifos test was inconsistent with the results obtained by Rust and Reierson (1976). Mortality was slightly decreased when compared with the chlorpyrifos alone although the decrease was not significant. Strong et al. (1998; unpublished data) demonstrated that lipids present in German cockroach fecal material can bind with several spray formulation toxicants including chlorpyrifos, making the active ingredient unavailable to cockroaches. The limited performance of the chlorpyrifos when combined with our

methanol extract in this test was suspected to be the result of the active ingredient becoming bound with the fatty acids and lipids present in the extract.

Both Ebeling et al. (1967) and Rust and Reierson (1996, 1977) found that boric acid powder was not enhanced by aggregation pheromone and concluded that this was because the powder was so non-repellent that the cockroaches continuously exposed themselves to the toxicant whether the pheromone attractant was present or not. In this study, the results of the boric acid/extract combination tests were less conclusive. Both of the extracts significantly enhanced the boric acid at 15 hours. However, enhanced mortality alternated between the aqueous extract, which produced significantly greater mortality 24 h, and the methanol extract which significantly enhanced mortality at 72 h. There were no differences in mortality between the two extract/boric acid combinations throughout the test period indicating that the presence of fecal extract did have some enhancement effects. Yet, further study is needed before the pheromone influence on non-repellent toxicants can be fully determined.

Pheromone usage in integrated pest management programs has developed in three main ways: mating disruption, mass trapping and insect population monitoring (Campion 1984). German cockroach pheromone use in an urban pest management program would take a completely novel approach. The

pheromone (fecal extract) would be applied within human living space and would function as a synergist for existing control techniques. However, for such an application to be acceptable the extract must be formulated in such a way that it is compatible with the human environment, easy to use, nontoxic and unobtrusive.

The aqueous pheromone extract used in this study presents several advantages for use in urban environment. The water based formulation is nontoxic and can be mixed easily with water based insecticide formulations. Because it has no color it can be used on all surfaces normally treated with residual formulations. Also, the aqueous extract can be used to enhance less toxic control methods such as bait stations and monitoring traps as well as the more repellent and toxic spray formulation insecticides.

Table 3-1. Mean mortality ± SEM of German cockroach nymphs exposed to chlorpyrifos alone, chlorpyrifos combined with fecal extracts, the aqueous extract or water.

Treatment	n	Mortality ± SEM		
		15 h	24 h	72 h
Chlorpyrifos + aqueous extract	8	62.7 ± 7.0a	75.9 ± 5.8a	78.6 ± 5.9a
Chlorpyrifos	8	28.3 ± 12.9b	40.5 ± 14.1b	46.1 ± 13.3b
Chlorpyrifos + methanol extract	8	19.7 ± 9.7b	25.3 ± 10.8b	28.1 ± 11.2b
Aqueous extract	8	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
Water	8	0.0 ± 0.0c	1.0 ± 1.0c	1.0 ± 1.0c

Analysis of variance: $F = 12.4$; df = 4; $P < 0.05$ (15 h mortality); $F = 17.3$; df = 4; $P < 0.05$ (24 h mortality); $F = 18.3$; df = 4; $P < 0.05$ (72 h mortality). Means followed by the same letter are not significantly different (Fisher's Test of Least Significant difference, critical $P \leq 0.05$ [SAS Institute 1988]).

Table 3-2. Mean mortality ± SEM of German cockroach nymphs exposed to boric acid alone, boric acid combined with fecal extracts, the aqueous extract or water.

Treatment	n	Mortality ± SEM		
		15 h	24 h	72 h
Boric acid + aqueous extract	8	19.9 ± 3.1a	42.3 ± 6.0a	74.8 ± 5.6ab
Boric acid + methanol extract	8	20.1 ± 4.4a	37.9 ± 5.4ab	82.4 ± 5.5a
Boric acid	8	6.0 ± 2.5b	25.1 ± 6.3b	65.0 ± 9.3b
Aqueous extract	8	4.1 ± 1.4c	4.1 ± 1.4c	7.8 ± 2.3c
Water	8	1.0 ± 1.0c	1.3 ± 1.0c	2.5 ± 1.3c

Analysis of variance: $F = 14.1$; df = 4; $P < 0.05$ (15 h mortality); $F = 21.8$; df = 4; $P < 0.05$ (24 h mortality); $F = 44.9$; df = 4; $P < 0.05$ (72 h mortality). Means followed by the same letter are not significantly different (Fisher's Test of Least significant difference, critical $P \leq 0.05$ [SAS Institute 1988]).

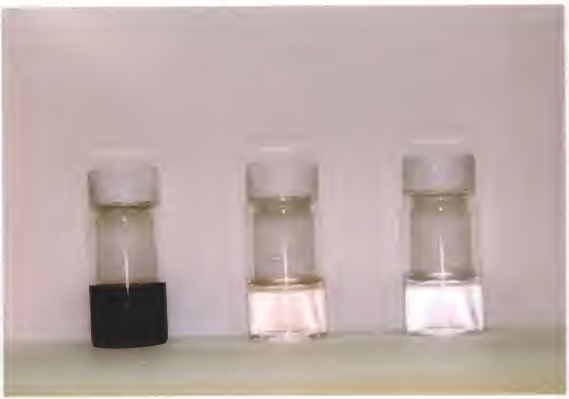


Figure 3-1. Comparison of fecal extracts, methanol extract (left) and aqueous extract (middle), with tap water (right).

CHAPTER 4
TRAIL FOLLOWING BEHAVIOR IN THE GERMAN COCKROACH (*Blattella germanica*)

Introduction

The use of chemical trails for directional orientation is widespread among social insects. The use of trail pheromones is best known among the ants, which use short-range chemoreception to detect trails of volatile and relatively non-volatile chemical components (Blum 1974). These trails are used for recruitment and directional orientation between sources of food and the nest (Blum 1974, Holldobler and Wilson 1990). Trail pheromones are also used extensively by termites to recruit workers for nest repair and for foraging in total darkness (Ebeling 1978). Butler et al. (1969) demonstrated that both honey bees and wasps lay passive trails with their "footprints". Secretions from dermal glands in the tarsi are deposited around the nest site orienting workers to the nest entrance.

Although insects that exhibit trail following behavior (e.g. ants and honey bees) may use physical landmarks and visual cues for directional orientation during the daylight hours (Klotz and Reid 1992), directional response to odors and chemical stimuli allow for free movement in the dark.

Non-social insects like the gregarious Eastern tent caterpillar use short range and tactile chemoreception at night to navigate between their foraging areas and the shelter of their tent (Fitzgerald and Gallagher 1976; Fitzgerald and Edgerly 1979, Peterson and Fitzgerald 1991).

The German cockroach is a gregarious, nocturnal insect whose directional locomotory behavior is influenced by chemical compounds found in their fecal material (Wendler and Vlatten 1993). There has been no documentation of German cockroaches using pheromone trails for directional orientation. However, Bell (1991) indicated that by observing the insect movement path we can determine the attention that cockroaches give to directional cues in their environment.

As early as 1974, Kitamura et al. suggested that German cockroaches leave and return to their harborages via the direction of chemical substances contained in their feces. Additionally, Sakuma and Fukami (1985) documented positive chemo- and anemo-taxes in German cockroaches toward frass-contaminated shelters. These two studies in addition to Wendler and Vlatten (1993) suggest that German cockroaches orient toward and follow paths of their fecal material.

The purpose of this study was to investigate German cockroach trail following behavior. The following experiments were carried out to evaluate the path of German cockroach movement in relation to a "trail" of fecal

extract. Additionally, I wanted to determine if trail following behavior differed among cockroaches of different age, sex and reproductive status. The amount of fecal extract necessary to elicit the trail following response was also investigated.

Materials and Methods

Cockroaches. Orlando strain German cockroaches (adult males, adult females and late instars) were obtained from the University of Florida Urban Entomology Laboratory in Gainesville, Florida. Cockroach rearing was conducted under the regimen outlined by Koehler and Patterson (1986) at 26° C, 55% RH and a photoperiod of 12:12 (L:D) h.

Fecal extract preparation. Feces from the Orlando strain German cockroaches were collected from the bottom of mass rearing containers. Ten grams of feces were separated from extraneous debris by sieving the fecal pellets with a No. 20 (0.84 mm) sieve. Sieved feces (10 g) were added to 20 ml of methanol to create a 1:2 w:w, feces:methanol mixture. The feces preparation was held in an Erlenmeyer flask (250 ml) and sealed with parafilm (American Can Company, Greenwich, CT) for 24 h. The supernatant was vacuum filtered through Whatman No. 1 filter paper in a Buechner funnel, and the filtrate was stored in a capped scintillation vial at 0° C.

Trail following arena. To minimize visual stimuli all tests were conducted under red filtered lighting in an empty

arena (1.4 m sq; 30.5 cm deep). The arena walls were extended to a height of 1.5 m. The floor and walls were covered in white waxed butcher paper (Georgia Pacific, Atlanta, GA). A test field in the middle of the arena floor was enclosed within a greased white plexiglass frame (1 m^2, 16 cm high) so that the cockroaches had no access to shadowed areas. A low light video surveillance camera (Model 4810 series monochrome solid state CCD; Cohu Inc. Electronics Division, San Diego, CA) was mounted at the top of the arena at 1.5 m above the test surface to capture cockroach movement.

Bioassay design. Strips of chromatography paper that had been treated with fecal extract were used to evaluate cockroach directional orientation and trail following behavior. Chromatography paper (Whatman International Limited, Kent, England) was cut into uniform strips (57 x 0.6 cm) with a paper shredder (Fellows Powershred PS50, Itasca, IL) and stored in an airtight Ziploc freezer bag (17 x 21 cm; Dowbrands, Indianapolis, IN). In preparation for each bioassay two strips of chromatography paper were individually wound into tight cylinders and placed inside watch glasses (3 cm diameter, 1 cm depth). The fecal extract (0.5 ml) was applied to one of the paper cylinders. The second cylinder was treated with an equal amount of methanol as a control. Each paper cylinder was then removed

from the watch glass with soft forceps (BioQuip Products, Gardena, CA), unwound, and hung to dry for 10 minutes.

During the drying period, 10 cockroaches (either adult male, female or nymph) were removed from rearing containers with featherweight forceps (BioQuip Products, Gardena, CA) and placed in individual harborages. Cockroach harborages were constructed by punching an exit hole in the side of an inverted plastic souffle cup (29.6 ml; Solo Cup Company, Urbana, Ill.) with a standard hole punch. The cup was then taped to an index card (7.6 x 12.7 cm; Mead Corporation, Dayton, OH) that had been cut in half. A second souffle cup was punched and placed over the first. The holes in the two cups were aligned for cockroach insertion. After the cockroach was put into the harborage, the holes were misaligned to prevent cockroach escape.

After the 10-minute drying period, the control and experimental test strips or "trails" of chromatography paper, were arranged in a V-shaped configuration and taped (3M Scotch Tapes, St. Paul, MN) to the floor of the test arena. For each replicate of the test, a harborage was placed at the vertex of the V and opened. The cockroach was allowed to exit and follow a course of its own choosing. After exiting the harborage cockroach movements were captured on a low light video surveillance camera and recorded as a time series of xy coordinates (one pixel = 2.5 cm) on an Apple Quadra 660AV computer. The recording of

cockroach movement was terminated either (a) when the cockroach ran out of the camera focal area, (b) the cockroach had completed running the length of one of the trails, or © when 45 seconds had passed since the cockroach exited the harborage.

Cockroach movement data were collected and recorded by the Dynamic Animal Movement Analyzer (DAMA; University of Florida Gainesville, FL) program for the Macintosh developed by Hoy (1994). The tracking parameters were set to record a moving object with a minimum pixel width of 2, and a maximum of 5. The minimum area scanned to locate the moving object was set at a minimum of 4 pixels with a maximum of area of 10. The grey threshold was set at 32, and 7.5 frames per second were recorded. The recording time was set at 0.75 min.

After the completion of 10 replications the paper lining the floor in the arena was replaced, and new trails of chromatography paper (one treated and one control) were placed in reversed positions from the previous replication. Each trail was bioassayed 10 times in each position for a total of 20 replicates from each cockroach group [i.e., adult males, adult females, late instars, gravid females].

The control bioassays were conducted as described for the fecal extract with the exception that both strips of chromatography were treated with methanol only. Each control bioassay had 10 replicates per cockroach group.

Statistical Analysis. The trail following ability of a particular cockroach group was assessed by measuring the perpendicular distance of each xy coordinate from the extract trail. These distances were averaged to assign each cockroach a mean distance value and standard deviation. The mean distance values facilitated analysis by eliminating over representation of the slow moving cockroaches in each group. Mean distance from the trail was analyzed with the Student's *t*-test for comparing independent samples; values of $P < 0.05$ indicated significance (SAS Institute 1988).

Mean values for trail following among cockroach life stages were analyzed by analysis of variance (ANOVA); values of $P < 0.05$ indicated significance. Where a significant *F* value was obtained, means were separated by Fisher's test of least significant difference (LSD) (SAS Institute, 1988).

Trail following TC_{50}. Serial dilutions were made of German cockroach fecal extract to determine the dose necessary to stimulate 50% of adult male cockroaches to follow the trail (TC_{50}). The extract was diluted 10x, 25x, 50x, 75x, and 100x with methanol (final extract concentrations: 10%, 7.5%, 5%, 2.5%, and 1%, respectively) in glass scintillation vials. Each dilution was formulated just prior to bioassay to avoid possible degradation of the active components in the extract.

Dose response tests were conducted in an empty portable arena (plastic rectangular tray; 56 x 43 x 10 cm).

Chromatography paper (Whatman International Limited, Kent, England) was cut into strips (39 x 0.06 cm) using a paper shredder (Fellows Powershred PS 50, Itasca, IL). The "trails" were stored and prepared for bioassay as described for the trail following bioassay. The extract dilution (0.5 ml) was applied to one of the paper trails. A second trail was treated with an equal amount of methanol. The trails were then hung until dry (10 min). While the trails were drying, cockroaches were prepared for bioassay as described above.

When completely dry, the paper trails were arranged in a V-shaped configuration and taped (3M Scotch Tapes, St. Paul, MN) to a glass pane (46.8 x 34 cm; 0.32 cm thick) on the floor of the test arena. The arena was covered with a plexiglass sheet (57 x 43 cm; 0.6 cm thick) overlaid with a Roscoe No.10 yellow gel to eliminate air movement and filter out wavelengths of light (including UV) that are visible to cockroaches (Koehler et al. 1987). For each run of the test a harborage was placed at the vertex of the paper trails and opened (Figure 4-1). The cockroach was allowed 5 minutes to exit the harborage and follow a course of its own choosing. The number of cockroaches that followed the trail of fecal extract (traveled within one antenna's distance of the trail along the entire length) was recorded. After each replicate of ten cockroaches the paper trails were discarded and the glass bottom of the arena was removed and cleaned with soap,

water, and acetone. The cleaned glass was then put back into the arena for the next replicate and the position of the trails was reversed. Each dose was replicated 10 times.

Statistical analysis trail following (TC_{50}). The TC_{50} for cockroach trail following response was estimated by probit analysis (SAS Institute 1988). Values of $P > 0.05$ were used to determine goodness-of-fit.

Results

The trail following data for each German cockroach group is illustrated in Figure 4-2(a-h). The black line indicates the position of the trail relative to the plot of each cockroach's movement, shown in grey.

Trail following bioassay. The comparisons of mean perpendicular distance from the trail of fecal extract (trail following behavior) versus the control are shown in Table 4-1. For each of the cockroach groups the mean perpendicular distance from the trail of fecal extract was significantly less ($P < 0.05$) than the distance from the control trails.

The analysis of variance indicated that trail following varied among the four cockroach groups (Table 4-2). The adult male mean perpendicular distance from the fecal extract trail (7.38 pixels) was significantly less than that of the late instars and gravid females. However, the mean male distance from the trail was not significantly less than that of the females (16.02 pixels). Mean distance between

females and late instars (18.75 pixels) also was not significantly different. Gravid females proved to have the poorest trail following ability. The mean perpendicular distance of the gravid females from the extract trail was 44.02 pixels. This was significantly greater than all other cockroach groups tested.

Trail following TC_{50}. German cockroach trail following response to varying concentrations of fecal extract are shown in Figure 4-3. The calculated TC_{50} for the adult male German cockroaches was relatively low. The concentration of fecal extract necessary to induce trail following behavior in 50% of adult male German cockroaches was 5.6% extract in methanol (n = 100; slope $\pm$ SE, 2.50 $\pm$ 0.38; 95% CI, 4.8%-6.5%; χ^2, 3.92; P = 0.14).

Discussion

The results of this study indicate that the paths of German cockroach movement are indeed influenced by chemicals found in their fecal material. Further, a relatively low concentration of fecal extract (and subsequently lower concentration of pheromonal chemicals) is required to elicit the trail following response.

The superior trail following ability of adult male cockroaches when compared with gravid females is consistent with differences observed in their general foraging frequency (DeMark 1992). Male German cockroaches forage more than any other cockroach life stage. Males were

observed making at least one foraging trip per day, seven days out of ten. Gravid females were shown to forage only three days out of ten. Females and nymphs fall in between males and gravid females in the number of foraging excursions that they make in search of food or water (DeMark 1992). This relationship between trail following ability and the number of foraging trips suggests that cockroach foraging may be directed by pheromonal cues. Within a given population, the males forage the most and are therefore the most sensitive to navigational cues in their environment. In contrast, the gravid females rarely leave the harborage and are subsequently the least influenced by chemical directional cues.

An interesting evolutionary model for trail following behavior in social insects was suggested by Maschwitz (1975). This model is also well-suited for German cockroaches because of their well documented loyalty to harborages and foraging areas contaminated with fecal material (Denzer et al. 1988). Maschwitz (1975) suggested that trail following insects (ants) would defecate outside the nest in particular places that were frequented by conspecifics. The characteristic smell was then an ideal predisposition for signaling highly frequented places like shelters and food sources. Subsequently, odorous chemicals in the feces easily took on an orientation function. Holldobler (1984) went further to argue that the use of the

hindgut contents as trail pheromones in formicine ant species evolved from a gradual ritualization of the defecation process.

I do not suggest that German cockroaches have a ritualized defecation process as do social insects. Instead, a more passive predisposition for signaling like the one described by Maschwitz (1975) is probably mediating German cockroach trail following behavior. In established field populations, chemical trails would develop over well traveled routes where pheromones from the cockroach bodies and fecal pellets tend to accumulate. These routes would be well defined between harborages and regular sources of food and water. Established members of the population would be able to use and contribute to these trails during their foraging excursions. Additionally, since these trails are already available, new additions to the population either by birth or immigration would not have to locate resources individually but simply follow in the foot steps of their conspecifics. Therefore, the trail following behavior would greatly enhance the survival and fitness of individual cockroaches by reducing the amount of energy expended to locate resources in their environment.

Table 4-1. Comparison of mean perpendicular distance (pixels) from fecal extract trail (trail following ability) versus control trails for different German cockroach life stages.

Cockroach	Treatment	n	Mean Perpendicular Distance ± SEM	*t*-value	*P*-value
Male	Fecal extract	20	7.38 ± 1.93		
	Control	10	48.01 ± 10.15	3.93	0.003
Female	Fecal extract	20	16.02 ± 3.60		
	Control	10	63.16 ± 14.41	3.17	0.010
Gravid	Fecal Extract	20	44.02 ± 7.84		
	Control	10	73.17 ± 5.55	3.03	0.007
Late Instar	Fecal Extract	20	18.75 ± 3.57		
	Control	10	73.75 ± 14.01	3.80	0.003

Two-tailed Student's t-test; $P \leq 0.05$ (SAS Institute 1988).

Table 4-2. Comparison of trail following accuracy (perpendicular distance in pixels from extract trail) for different German cockroach groups.

Cockroach Life stage	df	Mean Perpendicular Distance ± SEM	*F*	*P*-value
Male	3	7.38 ± 1.93a	16.47	0.0001
Female		16.02 ± 3.60ab		
Late Instar		18.75 ± 3.57b		
Gravid		44.02 ± 7.84c		

Analysis of variance (ANOVA):$P<0.05$. Means followed by the same letter are not significantly different (Fisher's test of Least Significant Difference; critical $P \leq 0.05$ [SAS Institute 1988]).

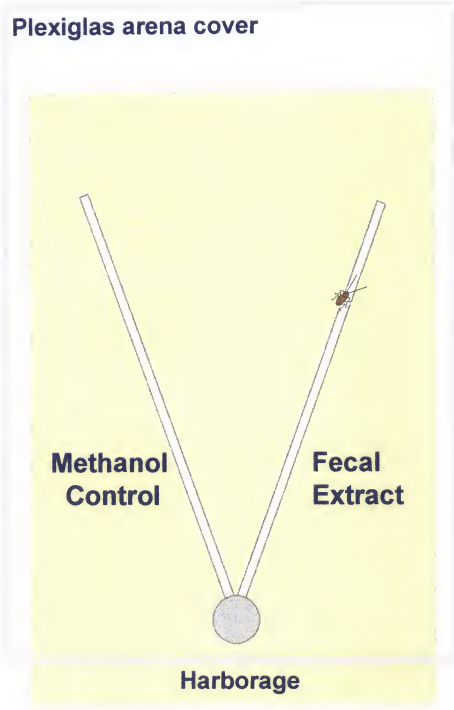


Figure 4-1. Diagram of arena for bioassay of German cockroach TC50.

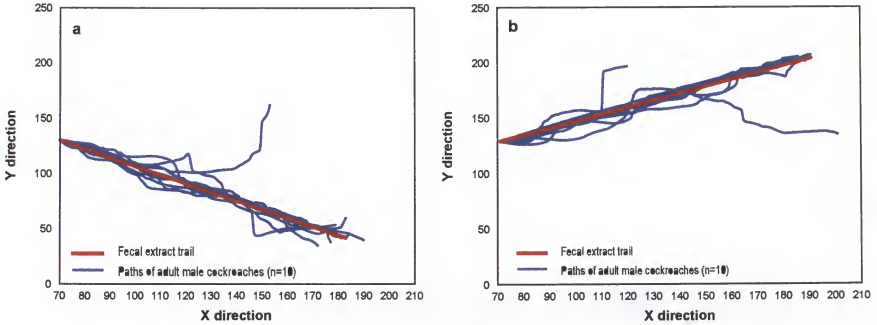


Figure 4-2. Plot of German cockroaches (in pixels) following a trail of fecal extract: a. Adult males following a trail to right, b. Adult males following a trail to left, c. Adult females following a trail to right, d. Adult females following a trail to left, e. Late instars following a trail to right, f. Late instars following a trail to left, g. Gravid females following a trail to right, h. Gravid females following a trail to left.

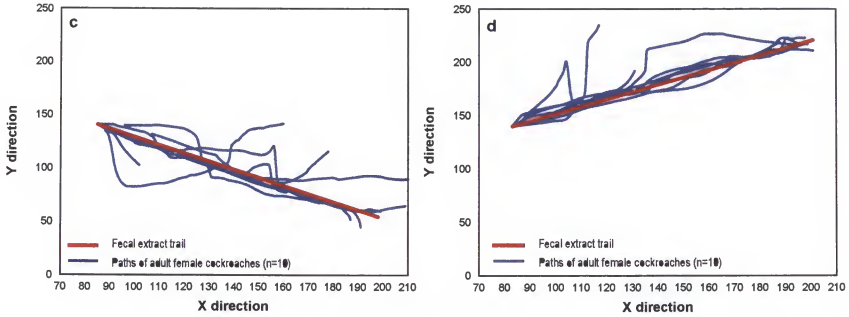


Figure 4-2 (continued).

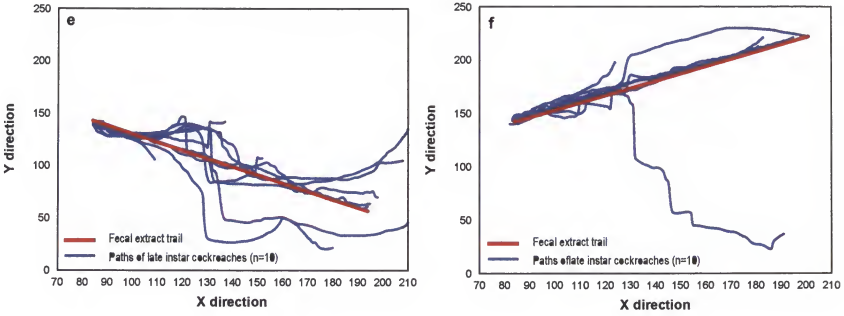


Figure 4-2 (continued).

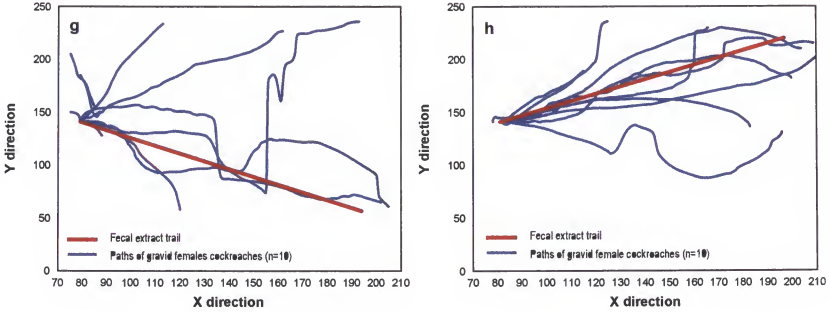


Figure 4-2 (continued).

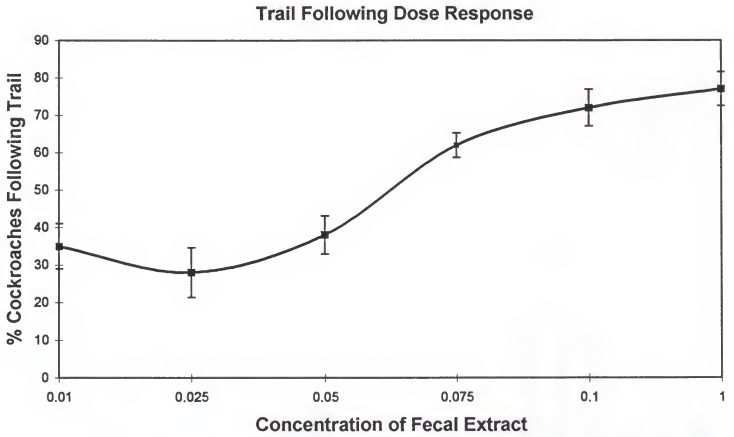


Figure 4-3. Concentration of German cockroach fecal extract which will induce trail following behavior in 50% of adult male cockroaches, TC_{50}=0.056 (SAS Institute, 1988).

CHAPTER 5
THE USE OF FECAL EXTRACT TRAILS TO ENHANCE TRAP CATCH IN GERMAN COCKROACH MONITORING STATIONS

Introduction

Pheromonal chemicals in German cockroach fecal material are known to induce cockroach aggregation behavior (Ishii 1970). Because of this pheromonal activity, extracts of cockroach feces have been used to evaluate the enhancement of several control techniques used in urban pest management programs.

Rust and Reierson (1976, 1977) were the first to document enhanced mortality for residual insecticides that were combined with a German cockroach fecal extract. The presence of the extract caused cockroaches to remain on treated surfaces longer causing them to pick up lethal doses of insecticide. Miller et al. (1996) demonstrated that bait stations were more attractive to German cockroaches when an extract of their fecal material was located inside. Further, the efficacy of toxic baits was significantly improved in the presence of competing non-toxic food sources when a fecal extract was placed next to a toxic bait inside a bait station (Miller 1997). Cockroach frass has been found to be such an effective attractant that one commercial

company (Woodstream Co., Lititz, PA.) has incorporated this material into sticky traps used for cockroach monitoring and control (Goldstein 1997).

In spite of its ability to improve pest management techniques, German cockroach fecal extract has had limited potential for use in the urban environment due to its unpleasant color and odor. These unpleasant characteristics make fecal extract fairly useless unless it is contained inside traps, such as the Woodstream product, or bait stations.

However, the foul color and odor associated with most fecal extracts can be eliminated by the process described in Chapter 1. In this process the fecal material is first extracted in methylene chloride and then the supernatant is mixed and separated with water. The resulting extract is suitable for use indoors. This aqueous formulation of German cockroach fecal material exhibits the aggregation pheromone type activity and has been shown to enhance mortality when combined with residual blatticides (Chapter 3).

Indoor monitoring traps and bait stations have a small physical presence in the environment, particularly in situations where the level of sanitation is low. Alternative food and harborage resources (Gupta et al. 1973, Bennett 1984) make baits and traps less effective. In a cluttered environment cockroaches may never discover a

monitor or bait station even if an attractant is located inside. Pest management efforts in these situations become more labor intensive. Ideally, pest control professionals would like to maximize the presence of these traps and bait stations so that their pest management efforts affect more of the cockroach population.

Reviews of pheromone applications have discussed the potential of pheromones being used as lures to attract insects into treated areas (Silverstein 1981). A pheromonal lure would help to enlarge the area influenced by a particular trap or bait placement. Cockroaches encountering the active space of the lure would be more likely to enter the trap or bait station and be eliminated from the population.

In Chapter 4, German cockroach directional orientation and trail following behavior was documented in response to extracts of their fecal material. This discovery suggests the possibility of using fecal extracts to lure German cockroaches from their harborages into traps or bait stations. However, the aqueous extract of German cockroach fecal material has never been tested for stimulating the trail following response.

The purpose of this study was to evaluate the aqueous formulation of the German cockroach fecal extract for trail following activity and determine its residual activity using two different storage methods. Additionally, the influence

of extract trails on trap catch was determined in arena tests where artificial trails were positioned between cockroach harborages and monitoring stations. The ratio of adult to nymph trap catch was also determined for both the extract trail treated traps and the controls.

Materials and Methods

Fecal extract preparation. Feces from the Orlando strain of German cockroaches were collected from the bottom of mass rearing containers. Feces (20 g) were separated from extraneous debris by sieving the fecal pellets with a No. 20 (0.84 mm) sieve. Sieved feces (20 g) were added to 40 g of methylene chloride for a 1:2 wt:wt feces:methylene chloride mixture. The fecal preparation was held in a capped 125-ml Erlenmeyer flask for 24 h. The supernatant was vacuum filtered through a Whatman No, 1 filter paper in a Buechner funnel. The filtrate was transferred into a 500-ml separatory funnel and shaken with an equal volume of water. The methylene chloride/water mixture was allowed to separate overnight. The clear aqueous phase was then removed with a micropipette and stored in a capped amber wide-mouth glass bottle (250 ml; Fisher Scientific Pittsburgh, PA) at 7°C.

Cockroaches. Orlando strain German cockroaches were obtained from the University of Florida Urban Entomology Laboratory in Gainesville, Florida. Cockroach rearing was conducted under the regimen outlined by Koehler and

Patterson (1986) at 26° C, 55% RH and a photoperiod of 12:12 (L:D) h.

Trail Following Bioassay. To test trail following activity of the aqueous extract, strips ("trails") of chromatography paper were treated with either the aqueous extract or water as a control. Chromatography paper (Whatman International Limited, Kent, England) was cut into uniform strips (57 x 0.6 cm) using a paper shredder (Fellows Powershred PS50, Itasca, IL) and stored in an airtight Ziploc freezer bag (17 x 21 cm; Dowbrands, Indianapolis, IN). In preparation for each bioassay, two strips of chromatography paper were individually wound into tight cylinders and placed inside watch glasses (3 cm diameter, 1 cm depth). Using a micropipette, 0.5 mls of the fecal extract were applied to one of the paper cylinders. The second cylinder was treated with an equal amount of water as a control. Each paper cylinder was then removed from the watch glass with soft forceps (BioQuip Products, Gardena, CA), unwound and hung until completely dry (ca. 17 minutes).

During the trail drying period, groups of 10 adult male German cockroaches were removed from rearing containers with featherweight forceps (BioQuip Products, Gardena, CA) and placed in individual harborages. Cockroach harborages were constructed by punching an exit hole in the side of an inverted plastic souffle cup (29.6 ml; Solo Cup Company, Urbana, Ill.) with a standard hole punch. The cup was then

taped to an index card (7.6 x 12.7 cm; Mead Corporation, Dayton, OH) that had been cut in half. A second souffle cup was then punched and placed over the first with the holes aligned for cockroach insertion. After the cockroach was put into the harborage the holes were misaligned to prevent cockroach escape.

When the "trails" of chromatography paper, one treated with the aqueous extract and one control, were completely dry, they arranged in a V-shaped configuration and taped (3M Scotch Brands, St. Paul, MN) to the floor of a plastic test arena (rectangular tray; 56 x 43 x 10 cm). The arena was covered with a plexiglass sheet (57 x 43 cm; 0.6 cm thick) overlain with a Roscoe No.10 yellow gel to eliminate air movement and filter out visible and UV light. For each run of the test a harborage was placed at the vertex of the paper trails and opened (Figure 5-1). The cockroach was allowed 5 minutes to exit the harborage and follow a course of its own choosing. The number of cockroaches that followed the extract treated trail (traveled within one antenna's distance of the trail along the entire length), or the control trail were recorded. Control bioassays were conducted as described above except that both trails in the arena were treated with water only.

Aqueous extract residual activity. Twenty-four paper trails were treated with the aqueous extract and hung to dry. Six of the trails were then bioassayed for trail

following activity as described above. Nine of the remaining 18 trails were then stored hanging in the open air at 26°C, 55% RH and a photoperiod of 12:12, L:D. Three of these trails were bioassayed every two days for one week or until trail following activity declined to < 20%. The remaining nine trails were placed inside individual airtight Ziploc bags (15.51 x 8.25 cm; DowBrands, Indianapolis, IN) and stored in a refrigerator at 0°C. Three of the refrigerated trails were bioassayed on days 3, 7 and 14 to access trail following activity. Residual activity for each storage method was evaluated by day using the analysis of variance (ANOVA). Values of $P < 0.05$ were considered significant. Means were separated using Fisher's test of least significant difference.

Trap enhancement bioassay. Orlando strain German cockroaches were removed from rearing containers and anesthetized with CO_2. The cockroaches were separated into mixed groups of 50 (ratio 2:3, adults:nymphs; 10 adult males, 10 adult females and 30 late instars) and placed inside 235 ml paper can harborages (Media Co., Charlotte, N.C.). Four opposing exits (1 x 2 cm) had been cut around the perimeter of each paper can. These exits were taped (3M Scotch Brands, St. Paul, MN) closed to prevent cockroach escape. Each harborage was provided with a moistened cotton ball and closed before the cockroaches revived. The closed harborages were placed in the center of test arenas (122

cm^2; 30.5 cm depth) that were lined at the bottom with heavy kraft paper and greased on the sides (2:1, petroleum jelly:mineral oil) to prevent cockroach escape. The cockroaches were allowed to acclimate inside the harborages for 24 h.

After the acclimatization period, the strips or "trails" of heavy kraft paper (91.4 x 0.64 cm) were rolled into cylinders, placed inside watch glasses (3 cm in diameter and 1 cm depth) and treated with 2 ml of the aqueous fecal extract or water (control). The trails were then unwound and hung until completely dry (ca. 20 min).

After drying, one extract treated trail and one control were aligned with two of the opposing exits of each harborage. The trails led from the harborage opening through the center of the arena. At the arena wall the trails made a 90° turn either to the left or to the right. At the turn the trails continued along the arena wall to the opening of a sticky trap (Lo-Line Insect Trap with attractant pellet; AgriSense, Columbia, MD). Traps were located on opposite walls of the arena either in diagonal positions (Figure 5-2) or opposing positions (Figure 5-3). The trails were taped along their edges in several places to keep them in position but the ends nearest the harborage were left free. The bottom of the arena was filled with open, hinged plastic food containers (10 x 10 cm, 8 cm depth; Solo Co. Urbana, IL) to add complexity. A piece of

laboratory rat chow was placed on top of the harborage and the tape was then removed from all four harborage exits. The free ends of the trails were folded into the two harborage exits and cockroaches were free to leave the harborage and explore the arena. The photoperiod was set for 12:12, L:D and each test was begun near the end of the photophase (6:00 pm). Trap catch was recorded after 24 h and the trap enhancement bioassay had 16 replicates.

Statistical analysis: trap catch. The mean difference in total trap catch between the extract trail treatment and the control was analyzed using the Student's *t*-test (SAS Institute 1988). Values of $P \leq 0.05$ were considered significant. The ratio of adults to nymphs, as a percentage of trap catch, was analyzed both within and between treatments using the Student's *t*-test (SAS Institute 1988). Values of $P \leq 0.05$ were considered significant.

Results

Trail Following Bioassay. The trail following bioassay indicated that the aqueous extraction of German cockroach fecal material did stimulate trail following behavior in adult male cockroaches. The mean percentage of cockroaches following the trail of extract (74%) was significantly higher than the percentage of cockroaches that followed either of the two water treated trails (22%) in the control bioassay (Table 5-1).

Residual activity of the aqueous extract. The residual activity of the aqueous extract treated trails varied according to how they were stored. On Day 1, 67-70% of adult male German cockroaches followed the extract treated trails (Figure 5-4). Trails that were stored in the refrigerator continued to elicit trail following behavior with no decline in activity over the entire two week period. The residual activity of trails stored in the open air declined significantly after 3 days (40%). Trail following activity was further reduced by Day 5 (33%) but this was not significant. However, activity was significantly less on Day 7 than on Day 5 with only 23 percent of the cockroaches following the extract trail.

Trap enhancement bioassay. Extract treated trails leading cockroaches from their harborage into sticky traps significantly enhanced trap catch (Table 5-2). The mean catch in traps combined with extract treated trails was 28 cockroaches. The mean number of cockroaches caught in traps with water treated trails was 11.

The trap catch ratio of adults:nymphs for extract treatment and the control were not significantly different. The ratio of adults:nymphs was 1.11:1 in the extract treated traps versus 1.15:1 in the controls (Table 5-3).

Discussion

The results obtained from the trail following bioassay indicate that the aqueous formulation of the fecal extract

was just as effective at stimulating the trail following response as the methanol extract described in Chapter 4. The simplicity of the extraction process plus the colorless, low odor characteristics of the final product make the aqueous formulation suitable for use in the indoor environment.

The residual activity of the aqueous extract indicates that when the extract is stored in cool, airtight conditions it will last for at least two weeks after application with minimal degradation. Preliminary tests evaluating the residual activity of an unapplied aqueous extract stored for six months in a refrigerated amber glass jar elicited trail following activity in 80% of adult male cockroaches (Miller and Koehler, unpublished data). The residual activity of the refrigerated extract suggests that when properly stored the extract would have a long shelf life. However, the shelf life of the aqueous extract needs to be accurately determined before long term potential can be assessed.

Because we do not know the chemical content of the aqueous extract, we can only speculate as to the cause of its degradation when exposed to open air. One possibility is the evaporation of volatile attractants that are known to be present in German cockroach fecal pellets. Sakuma and Fukami (1990) identified several volatile amines in German cockroach frass that had attractant activity. The most attractive components were identified as alkylamines (Sakuma

and Fukami 1990). In cockroach fecal pellets these amines would be deposited on substrates not as free amines but as salts. These salts reduce the rate of evaporation and give these attractants greater stability (Sakuma and Fukami 1990). It is possible that alkylamines play a role in cockroach trail following behavior in the field. If so, the degradation in extract activity suggests that the natural amine salts present in the fecal material were hydrolyzed during the extraction process. This would explain the rapid evaporation of the attractant chemicals from the trails when they were stored in the open air. Sakuma et al. (1996ab) demonstrated that the addition of organic acids or hydrochloric acid to the free amines (thus forming a salt) controlled amine release and reduced the rate of evaporation. It is possible that the addition of HCL or an organic acid to the aqueous extract would extend the residual activity of the extract after it has been applied to a substrate. However, there are over 150 compounds that have been identified in cockroaches feces (Fuchs et al. 1985). It remains to be determined which of these, if any, are actually present in the aqueous fecal extract.

The use of monitoring traps is an essential part of any urban integrated pest management (IPM) program. Although adhesive traps should not be considered control devices it is still important that they capture a representative sample of the cockroach population (Owens and Bennet 1983). Often

pest control professionals use adhesive traps simply as cockroach detectors, in which case the entire trapping effort need only catch one cockroach to initiate control efforts. However, catching one or a small number of cockroaches provides very little information about the local cockroach habits and population foci. If the trap catch were more substantial, the pest control operator could determine the direction from which the cockroaches were traveling when they entered the trap by looking at the relative positions and orientation of the stuck cockroaches (Owens 1995). This information could then be used to locate cockroach harborages as well as sources of food and water. Subsequent control efforts could then be focused into these specific areas frequented by cockroaches.

Leading cockroaches into adhesive traps via fecal extract treated trails significantly enhances trap catch. In the field, extract trails would serve to increase the amount of physical space influenced by the placement of a single trap. This would be particularly beneficial in cluttered environments where cockroaches may never find a trap in the complex surroundings.

Although the total number of cockroaches captured was significantly increased by the addition of extract trails, the trapping effort was biased toward adult cockroaches. The beginning ratio of adults to nymphs was 20:30 (2:3). However, our adult to nymph catch ratio in both the treated

and control traps was practically 1:1 (1.15:1 and 1.11:1; no significant difference). Our bias for sampling adult cockroaches was identical to that presented by Moore and Granovsky (1983). Combined catch data for four commercially available adhesive traps found that trap catch ratios, adult:nymph, were nearly 1:1 (2.38:1.94) while the initial populations in their arena tests had an adult:nymph ratio of 40:60 (2:3). The enhanced trap catch and bias toward the capture of adult cockroaches in our tests indicated that the trails of fecal extract were more influential on the movement of adult cockroaches than the immatures. This observation is substantiated by the trail following ability data reported in Chapter 4.

In summary, the aqueous extract of German cockroach fecal material did elicit trail following behavior in German cockroaches. Residual activity of the extract did not decline when treated surfaces were sealed and refrigerated but when exposed to open air the trail following activity declined significantly after only three days. Although, the aqueous extract treated trails did not change the bias for trapping adult cockroaches, the presence of the trails did significantly enhance trap catch overall.

Table 5-1. Mean percent of adult male cockroaches following a trail treated with the aqueous fecal extract versus control trails treated with water only (n=5).

Treatment	Mean percent following trail ± SEM	*t*-value	*P*-value
Aqueous Fecal Extract	74.00 ± 4.0	6.71	0.0002
Water	22.00 ± 6.6		

Student's t-test; $P \leq 0.05$ (SAS Institute 1988).

Table 5-2. Mean difference in catch between traps that had trails treated with fecal extract leading to the entrances and traps that had trails treated with water only (control) (n=16).

Treatment	Mean trap catch ± SEM	Mean difference in trap catch ± SEM[a]	*t*-value	*P*-value
Aqueous Fecal Extract	28.1 ± 1.1	17.1 ± 1.7	10.16	0.0001
Water	11.0 ± 1.1			

Student's t-test; $P \leq 0.05$ (SAS Institute 1988).
[a]Mean difference in trap catch ± SEM was used to determine *t*-statistic and *P*-value.

Table 5-3. Ratio of adult:nymph cockroaches as a percent of catch in traps with the aqueous extract treated trail compared with the catch ratio in traps with the control trail (n=16).

	Mean percent trap catch + SEM[a]		Ratio
Treatment	Adults	Nymphs	Adults : Nymphs
Aqueous Fecal Extract	52.8 ± 9.8a	47.2 ± 9.8a	1.12 : 1
Water	53.5 ± 17.8a	46.5 ± 17.8a	1.15 : 1

[a]Student's t-test; $P \leq 0.05$. Means followed by the same letter (both within and between treatments) are not significantly different (SAS Institute 1988).

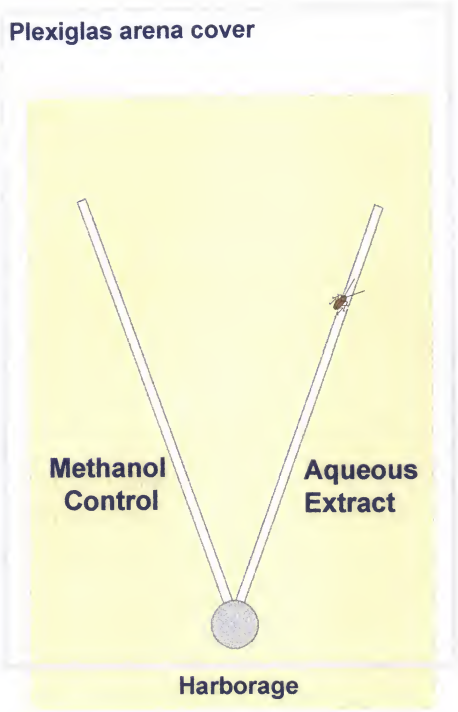


Figure 5-1. Diagram of arena for bioassay of German cockroach aqueous fecal extract.

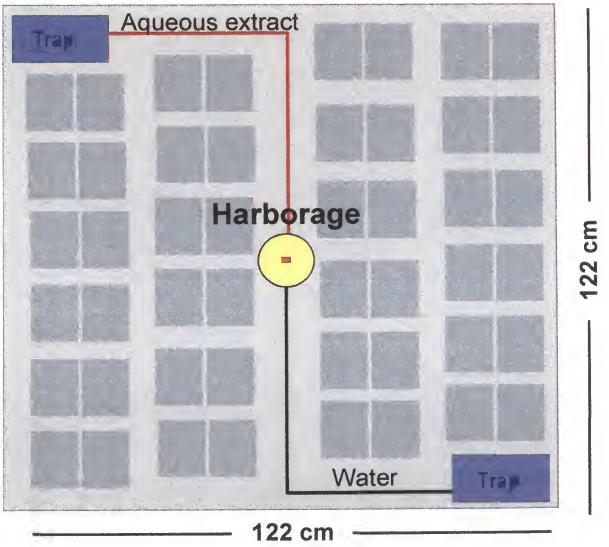


Figure 5-2. Diagram of bioassay arena with traps in diagonal positions.

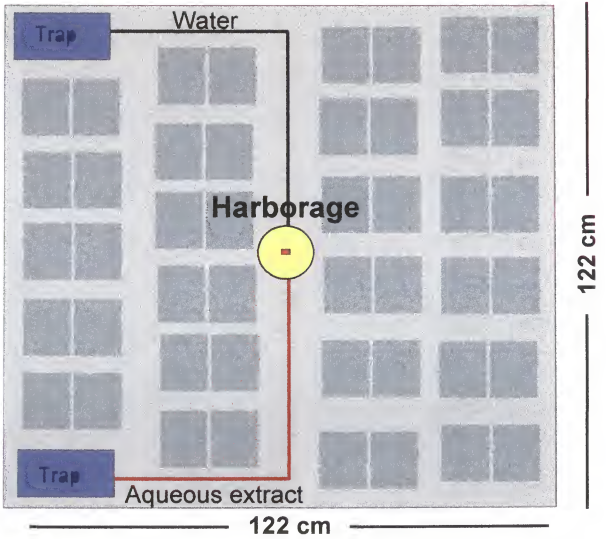


Figure 5-3. Diagram of bioassay arena with traps in opposing positions.

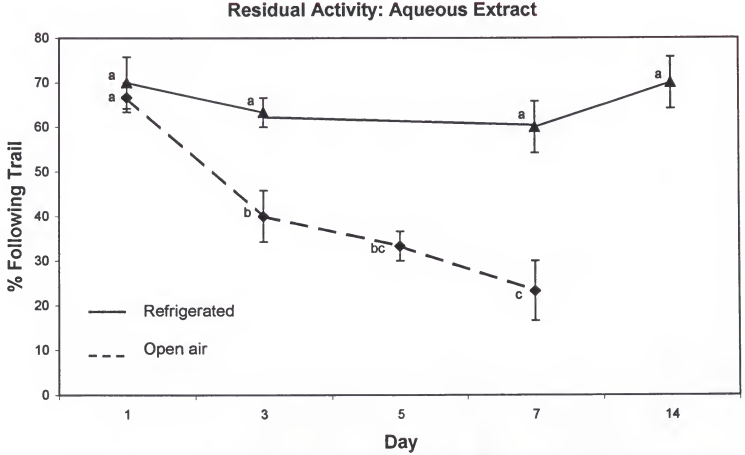


Figure 5-4. Residual activity of aqueous extract treated trails stored in the refrigerator versus trails stored in open air. Data points preceded by the same letter are not significantly different.

CHAPTER 6
ANALYSIS OF GERMAN COCKROACH FECAL EXTRACT: TRAIL PHEROMONE

Introduction

For the last 20 years the term "aggregation pheromone" has often been misapplied, referring to fecal extracts that are used to manipulate German cockroaches in scientific studies (Rust and Reierson 1976, 1977; Glaser 1980; Wendler and Vlatten 1993). Methanol extracts of German cockroach feces have been used to evaluate the influence of "aggregation pheromone" on different cockroach behaviors. Surface preference (Ogg 1989), food consumption (Miller 1997), harborage choice (Miller 1996) are but a few of the studies that examined cockroach behavior in the presence of an "aggregation pheromone."

Fecal extracts have also been used to evaluate aggregation pheromone effects on cockroach movement, specifically, directional orientation and locomotion. Kitamura et al. (1974) was the first to suggest that chemicals present in an extract of fecal material were responsible for German cockroach directional orientation. Wendler and Vlatten (1993) evaluated the influence of "aggregation pheromone" (a fecal extract) on directional walking behavior in German cockroach males. However, in all

of the above studies the actual chemical composition of the extracts and the quantity of pheromone present in the extracts was not known.

In 1990 Sakuma and Fukami determined that the odorous attractant components of German cockroach aggregation pheromone were a suite of amines. The most active attractant was identified as 1-dimethylamino-2-methyl-2-propanol or DMAMP: mass spectrum (significant *m/z* (relative intensity)) 117(8), 102(14), 100(3), 58(100), 44(11), 42(9) (Sakuma and Fukami (1990). The DMAMP was then synthesized and tested for attractant activity in an olfactometer assay. Like the extracted DMAMP the synthesized pheromone was found to be 50 to 1000 times more active than the other attractant components (Sakuma and Fukami 1990).

Three years later Sakuma and Fukami (1993) identified the arrestant components in aggregation pheromone. These were nonvolatile sterol glycosides that halted German cockroach forward movement when tactile contact was made. The attractant and arrestant components reportedly worked in concert to mediate aggregation behavior. The DMAMP attracted cockroaches causing them to move up the pheromone concentration gradient and then the glycosides halted their movement once contact was made with the pheromone source.

Kitamura et al. (1974) and Wendler and Vlatten (1993) related the directional orientation to short range antennal or tactile chemoreception. This would indicate that the

non-volatile arrestant components of aggregation pheromone might be related to cockroach trail following behavior. However, the cockroach forward movement is stimulated rather than arrested suggesting that the attractant DMAMP was the most likely known compound mediating the trail following behavior.

Although DMAMP attractant activity has been determined in linear olfactometer studies (Sakuma and Fukami 1985, 1990, 1996), it has never been evaluated for eliciting trail following behavior. Additionally, the amount of DMAMP in methanol extracts of German cockroach fecal material has never been quantified. The primary purpose of this study was to compare the trail following activity of the DMAMP (aggregation pheromone) identified by Sakuma and Fukami (1990) with a methanol extract of German cockroach feces. Additionally, we wanted to analyze the fecal extract for the presence of DMAMP using gas chromatography and mass spectrometry.

Materials and Methods

Cockroaches, Adult male German cockroaches (Orlando strain) were obtained from the University of Florida Urban Entomology Laboratory in Gainesville, Florida. Cockroach rearing was conducted under the regimen outlined by Koehler and Patterson (1986) at 26° C, 55% RH and a photoperiod of 12:12 (L:D) h.

Methanol extract preparation. Feces from the Orlando strain of German cockroaches were collected from the bottom of mass rearing containers. Twenty grams of feces were separated from debris by sieving the fecal pellets with a No. 20 (0.84 mm) sieve. Sieved feces (20 g) were added to 40 grams of methanol for a 1:2, wt:wt, feces: methanol mixture. The feces mixture was held in 125-ml Erlenmeyer flask sealed with parafilm (American Can Company, Greenwich, CT.) for 24 h at room temperature (24°C). The supernatant was vacuum filtered through a Whatman No.1 filter paper in a Buechner funnel, and the filtrate was refrigerated in a capped scintillation vial (20 ml) at 0°C.

DMAMP standard. A liquid sample of synthesized DMAMP (100 mg; Lot No. 18712) in a sealed glass ampule was obtained from the Nitto Denko Corporation in Osaka, Japan. The ampule was opened with a metal file and the contents transferred into an amber glass vial (2 ml; National Scientific Co. Norcross, GA.) with a Teflon coated seal cap (National Scientific Co. Norcross, GA.) and stored in a freezer at -43°C. Figure 6-1 is a gas chromatogram (Nitto Denko Corporation in Osaka, Japan) of the DMAMP produced on a DB-1 column (30 m x 0.25 mm; Helium 65kPA). The injection temperature was 250 °C with an oven temperature program beginning at 40°C with an initial hold time of 5 minutes increasing at 10°C/min to 200°C. The detector temperature was 250°C.

Trail following bioassay: methanol extract. To minimize visual stimulus, all tests were conducted in an empty arena (rectangular plastic tray; 56 x 43 x 10 cm). Chromatography paper (Whatman International Limited, Kent, England) was cut into uniform strips (39 x 0.06 cm) using a paper shredder (Fellows Powershred PS 50, Itasca, IL) and stored in an Ziploc freezer bag (17 x 21 cm; Dowbrands, Indianapolis, IN). In preparation for each bioassay two strips of chromatography paper were individually wound into tight cylinders and placed inside watch glasses (3 cm diameter and 1 cm depth). Using a micropipette, 0.5 ml of the fecal extract was applied to one of the paper trails. The second trail was treated with an equal amount of methanol. The paper trails were then removed from the watch glass with soft forceps (BioQuip Products, Gardena, CA), unwound and hung until completely dry (10 min).

While the trails were drying, 10 German cockroaches were removed from rearing containers with featherweight forceps (BioQuip Products, Gardena, CA) and placed in individual harborages. Cockroach harborages were constructed by punching an exit hole in the side of an inverted plastic souffle cup (29.6 ml; Solo Cup Company, Urbana, Ill.) with a standard hole punch. The cup was then taped to an index card (7.6 x 12.7 cm; Mead Corporation, Dayton, OH) that had been cut in half. A second souffle cup was then punched and placed over the first with the holes

aligned for cockroach insertion. After the cockroach was put into the harborage the holes were misaligned to prevent cockroach escape.

When completely dry, the paper trails were arranged in a V-shaped configuration and taped (3M Scotch Tapes, St. Paul, MN) to a glass pane (46.8 x 34 cm; 0.32 cm thick) at the bottom of the test arena. The arena was covered with a plexiglass sheet (57 x 43 cm; 0.6 cm thick) overlaid with a Roscoe No.10 yellow gel to eliminate air movement and filter out visible and UV light. For each run of the test a harborage was placed at the vertex of the paper trails and opened (Figure 6-2). The cockroach was allowed 5 minutes to exit the harborage and follow a course of its own choosing. The number of cockroaches that followed either trail (traveled within one antenna's distance of the trail along the entire length), or simply explored the arena were recorded. After each replicate of ten cockroaches the paper trails were discarded and the glass bottom of the arena was removed and cleaned with soap, water and acetone. The cleaned glass was then put back into the arena for the next replicate and the position of the trails was reversed.

Trail following bioassay: DMAMP. Chromatography paper (Whatman International Limited, Kent, England) was prepared as described for the methanol extract bioassay. The preparation of DMAMP for application on the paper trails was complicated by the volatility of the compound. Sakuma et

al. (1996a) reported DMAMP to be so volatile that complete evaporation would be expected immediately upon application to a substrate. The authors stated that controlled release of the DMAMP could be achieved by salt formation. In the cockroach feces the natural state of the amine was a salt formed with hydrochloric acid (HCl; Sakuma et al. 1996a). The pH of the amine salt was reported to be ~5.0 (Sakuma et al. 1996a). Based on this information, we combined the DMAMP with HCl in water to facilitate salt formation. Initially, 12.2 μls of synthesized DMAMP was diluted in 0.6 ml of water for a 2% concentration. However, a pH test using Litmus paper indicated that the resulting solution was extremely alkaline (pH 14), so an additional ml of water was added. To form the salt, 0.02 ml of HCl (1 Normal) was added to the solution. The resulting pH fell within the desired range of ~4.5 - 5.0. Using a micropipette, 0.5 ml of the DMAMP salt solution was applied to one of the paper trail cylinders. The second trail was then treated with an equal amount of water. The trails were then removed from the watch glass with soft forceps (BioQuip Products, Gardena, CA), unwound and hung until completely dry (~17 min). The trail following protocol was the same as described for the methanol extract.

GCMS analysis of DMAMP standard. One μl of the neat DMAMP standard was injected into a 25 m x 0.20 mm DB1 GC column (J&W, Folsom, CA) and analyzed on a gas

chromatograph/mass spectrophotometer (Hewlett Packard 5890 Series 2; Palo Alto, CA). The DMAMP injection was made in the splitless mode with an injection port temperature of 250°C. The GC oven was programmed from 40°C to 250°C increasing at 10°C/min with initial and final hold times of 5 minutes. Electron impact (EI) and chemical ionization (CI) mass spectra were acquired in the continuous-scan mode, scanning from *m/z* 50 to *m/z* 250 at ca. 2 scans per second.

GCMS analysis of methanol extract. A 3 μl-aliquot of the methanol extract was injected into a 25 m x 0.20 mm DB1 GC column and analyzed as stated above. The extract injection was made in the splitless mode with an injection port temperature of 250°C. The GC oven was programed from 40°C to 250°C increasing at 10°C/min with initial and final hold times of 1 minute. Electron impact (EI) and chemical ionization (CI) mass spectra were acquired in the continuous-scan mode, scanning from *m/z* 50 to *m/z* 250 at ca. 2 scans per second. In addition, selected ion monitoring (SIM) was used in an attempt to enhance the sensitivity and selectivity for DMAMP. Using the SIM mode of analysis the instrument was not scanned continuously over a given range of *m/z* values but was stepped between selected *m/z* values corresponding to ions characteristic of DMAMP. In this study the *m/z* values monitored were 58, 102, and 117.

Results

Trail following bioassay: methanol extract. The mean percentage of cockroaches following the trail of methanol extract was significantly greater than those that followed either of the control trails (Table 6-1). Sixty-eight percent of the adult male German cockroaches followed the trail of fecal extract while only 14% of the German cockroaches followed either one of the two paper trails in the control bioassay.

Trail following bioassay: DMAMP. Ten German cockroaches were assayed but only 20% followed the trail of DMAMP. These results were not significant because 20% of the cockroaches would follow one trail or the other when both were treated with water in a control bioassay. A second assay was performed with a new trail and again no significant trail following behavior was observed.

GCMS analysis DMAMP. The DMAMP standard produced a gas chromatogram with a major, overloaded peak at 5.9 min and a smaller peak at 6.4 min (Figure 6-3). This chromatogram was identical to the original chromatogram that was sent with the standard from Nitto Denko. As stated above, the electron impact (EI) spectrum of the major peak was also identical to that obtained in the Sakuma and Fukami (1990) study (Figure 6-4). The EI spectrum of the second peak was virtually identical to that of the major peak and was attributable to an isomeric contaminant.

The chromatogram obtained for the DMAMP standard under the chemical ionization (CI) conditions (Figure 6-5) displayed three peaks, the first was the solvent tail. The second and third peaks corresponded to the two peaks seen under the EI conditions (Figure 6-3). The spectrum of the major peak was dominated by the MH^+ ions at *m/z* 118 (Figure 6-6). Ions at *m/z* 146 and 158 corresponded to the $C_2H_5M^+$ and $C_3H_5M^+$ adducts which are frequently seen in CI spectra. As in the EI analysis the spectrum of the secondary peak was virtually identical to that of the major peak.

GCMS analysis: methanol extract. The gas chromatogram produced by the methanol extract is shown in Figure 6-7. A peak indicating the presence of DMAMP was not observed at the anticipated retention time of 5.9 min. The full-scan EI analysis of the methanol extract also produced a very rich chromatogram (Figure 6-8). However, the ions at *m/z* 58 and 117, which are diagnostic for DMAMP did not maximize at the retention time expected from the DMAMP standard.

In the analysis using selective ion monitoring (SIM) the sensitivity enhancement was a factor of ca. 100. The resulting SIM chromatogram was very rich even though only the *m/z* values of 52, 102 and 117 were monitored (Figure 6-9). This was because many compounds other than DMAMP produce ions with these *m/z* values. The numerous peaks near the retention time of the standard precludes assigning any of them unambiguously to DMAMP. This problem is intrinsic

to the low molecular weight of DMAMP (117) and the most prominent ion has a very low value of *m/z* 58, which is also seen in many interferents.

Analysis of the methanol extract under full-scan CI conditions yielded a much less cluttered chromatogram (Figure 6-10) but the characteristic *m/z* of 118 did not elute at a retention time near that of DMAMP standard (Figure 6-11).

Discussion

DMAMP as it was applied in our study failed to influence or attract German cockroaches. DMAMP was analyzed with the gas chromatograph mass spectrometer and the spectra produced was identical to that reported by Sakuma and Fukami (1990) confirming that no degradation of the chemical had occurred (Figure 6-4). In contrast our crude methanol extract did elicit the trail following response. The failure of DMAMP to stimulate trail following suggests that this attractant component of aggregation pheromone does not influence this behavior.

The fact that DMAMP could not be detected in the methanol extract using any of the available GC-MS techniques further indicates that aggregation pheromone does not mediate the trail following response. The neat sample of DMAMP was assumed to have a density of 1 g/mL. Thus it would have a lower limit of detection estimated as ca. 50 ppm using SIM under CI conditions. This detection limit

would be considered *very* approximate. A more accurate determination would require making dilutions determining the lowest concentration that can be detected with a signal-to-noise ratio of >3.0. From the data generated in this study it could be stated that DMAMP if present in the methanol extract had a concentration of <50 ppm. It is very likely that German cockroaches can detect concentrations of DMAMP <50 ppm but the specific behavior we were looking for in this test suggests that aggregation pheromone detection might not be a factor.

Aggregation behavior is a response to volatile chemicals that are detected by the antennae (Sakuma and Fukami 1990). The cockroach moves in the direction of the stimulus then stops when it comes in contact with the arrestant component of the pheromone. By contrast, trail following behavior is initiated by tactile contact with the fecal extract which stimulates the cockroach to continuously move along the extract trail (Chapter 4).

Wendler and Vlatten (1993) stated that when a cockroach contacted fecal extract on a strip of filter paper, the contact stimulated "aggregation in the "free state", meaning that the cockroach would walk in the direction of the strip. Wendler and Vlatten (1993) concluded that this directed movement combined with slower walking speed and occasional stops "must contribute to the process of aggregation" although they admit that this locomotory behavior was also

observed in other insects that were not aggregating. Further, Wendler and Vlatten (1993) assayed cockroaches individually and never documented the full cessation of movement. This suggests the authors' assertion, that the observed directional locomotion was a precursor to aggregation, was unfounded.

German cockroach response to extracts of their fecal material have heretofore been interpreted solely in terms of aggregation behavior. The widespread confusion of terminology, referring to fecal extract as aggregation pheromone, has led to this misinterpretation of cockroach behavior. Trail following is a documented cockroach response to extracts of their fecal material (Chapter 4). The fact that DMAMP, an essential component of aggregation pheromone, failed to stimulate the trail following response confirms that trail following and aggregation are two different behaviors.

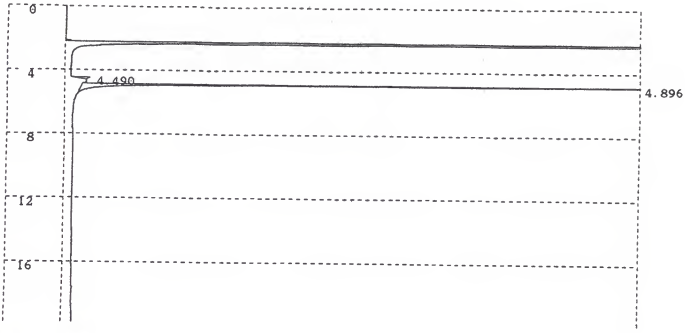
Based on the observation that aggregation behavior is independent of trail following behavior and that the DMAMP neither stimulated trail following nor was detectable in the active methanol extract, I propose that the active component responsible for the trail following behavior in German cockroaches is not aggregation pheromone but a different chemical stimulus or chemical combination. I offer to name this chemical(s) "German cockroach trail pheromone."

I acknowledge that the sterol glycosides or arrestant components of aggregation pheromone may still play a significant role in cockroach trail following. These components need to be bioassayed to fully understand their influence on cockroach behavior. However, if these components, blattellastanoside A or B (Sakuma and Fukami 1993) are found to stimulate trail following behavior they should no longer be termed "arrestants" in future literature.

Table 6-1. Mean percent of German cockroach males following a trail of methanol fecal extract or a trail of methanol alone.

Trail	n	Mean % ± SE cockroaches following trail	*t*-value	*P*
Methanol extract	10	68.00 ± 7.42	-6.31	0.0001
Methanol only	10	14.00 + 4.27		

Two-tailed Student *t*-test: *P*=0.05 (SAS Institute 1988).



** 定量計算結果 **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
2	1	4.49	2222	430			2.6736	
	2	4.896	80897	31127			97.3264	
		TOTAL	83119	31557			100	

Figure 6-1. Gas chromatogram of DMAMP on DB-1 column (Nitto Denko Corporation, Osaka, Japan).

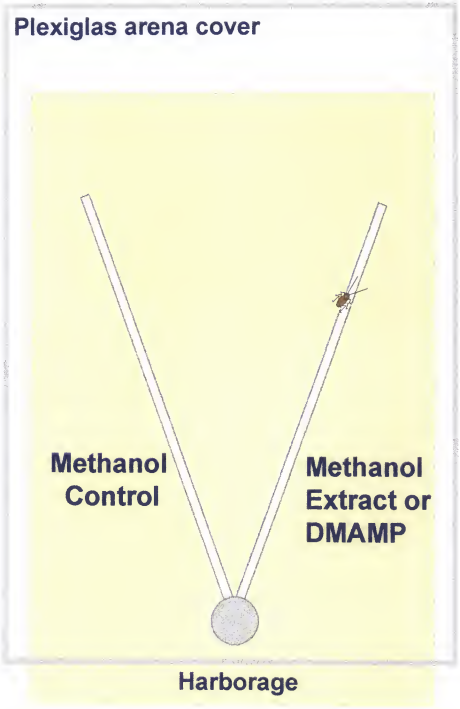


Figure 6-2. Diagram of arena for bioassay of German cockroach trail following behavior.

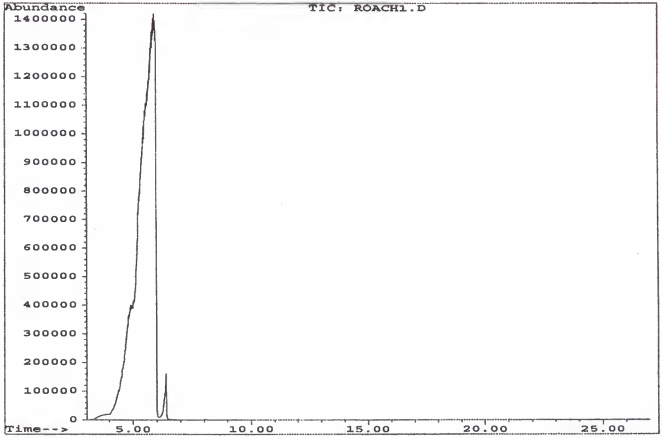


Figure 6-3. Gas chromatogram of DMAMP standard taken from DB-1 column (J&W).

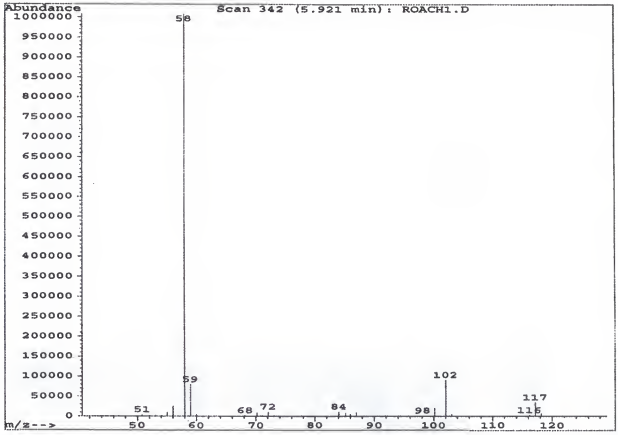


Figure 6-4. Electron impact analysis (EI) of major peak (5.9 min), DMAMP standard.

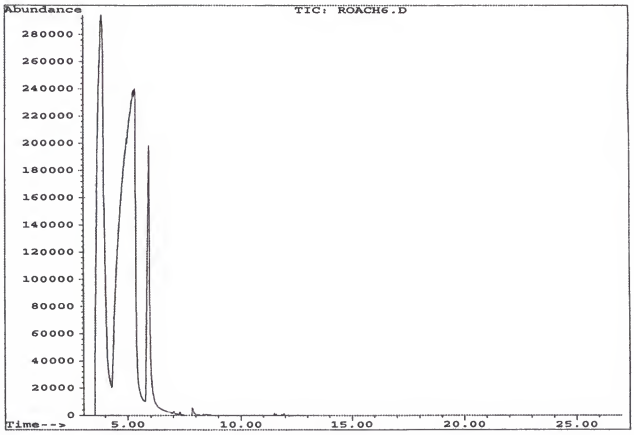


Figure 6-5. Chromatogram of DMAMP standard under chemical ionization conditions (CI).

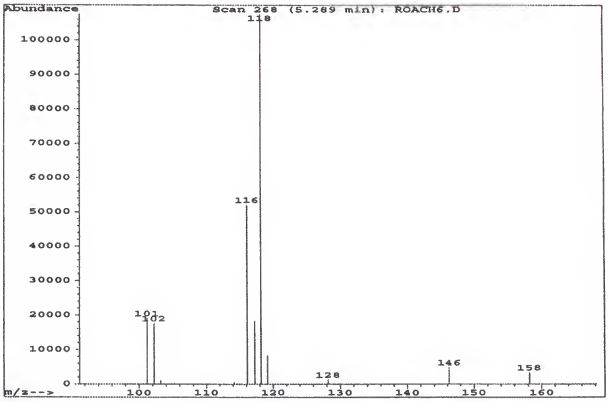


Figure 6-6. Spectrum of the major peak from DMAMP standard under chemical ionization conditions (CI).

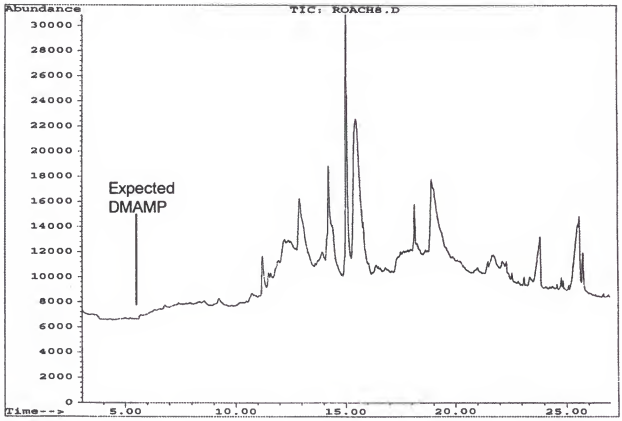


Figure 6-7. Gas chromatogram of methanol extract of German cockroach fecal material taken from DB-1 column (J&W).

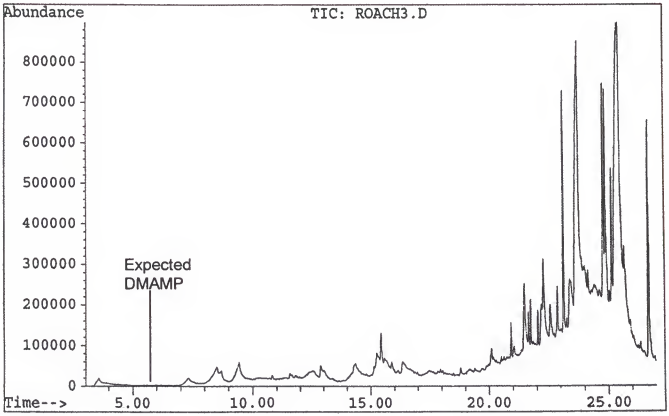


Figure 6-8. Full-scan electron impact (EI) analysis of methanol extract (German cockroach fecal material).

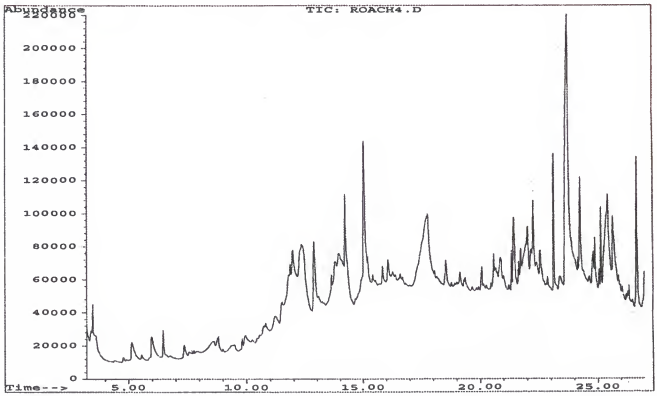


Figure 6-9. Gas chromatogram of methanol extract analyzed by selected ion monitoring (SIM) for m/z values of 58, 102, and 117.

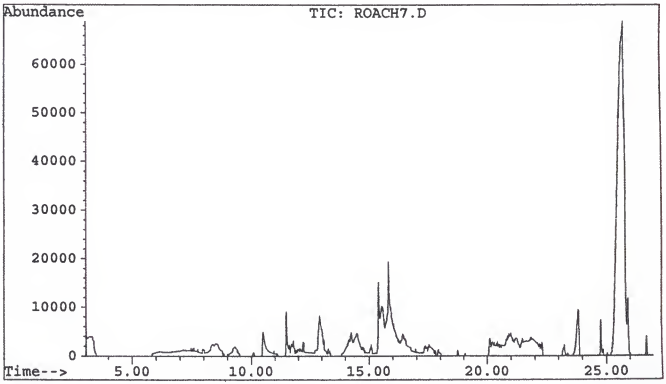


Figure 6-10. Chromatogram of methanol extract under full-scan chemical ionization (CI) conditions.

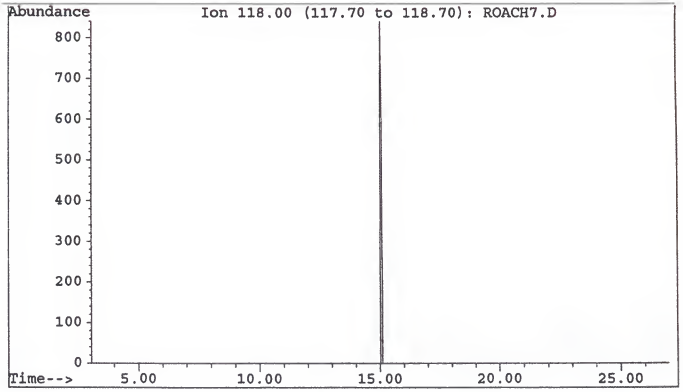


Figure 6-11. Spectrum of ion m/z 118 from the methanol extract under chemical ionization conditions (CI). The compound producing the ion does not elute from the GC column near the expected retention time of DMAMP. Thus, the ion m/z 118 does not originate from DMAMP in the methanol extract.

CHAPTER 7
SUMMARY

Ever year billions of dollars are spent in an effort to control German cockroach, *Blattella germanica* (L.) populations in urban structures. These pests are not only obnoxious and embarrassing but they also present a significant health risk to humans that share their environment. German cockroaches are able to mechanically transmit disease organisms on their bodies as well as in their feces. In infested situations the large quantities of frass produced by these insects can cause food contamination and respiratory allergic reactions.

German cockroach frass is highly undesirable in human living space yet very important to the cockroaches themselves. German cockroaches feces are an important source of nutrition for young nymphs. They also serve as territorial markers for identifying particular harborages and a home range. Additionally, volatile and nonvolatile chemicals present in German cockroach feces are responsible for their aggregation behavior.

Kitamura et al. (1974) suggested that German cockroach fecal pellets played a significant role in cockroach directional orientation while foraging. However, this

directional behavior had never been evaluated. Methanol extracts of German cockroach fecal material (termed "aggregation pheromone") were shown to influence the direction of walking behavior in adult males (Wendler and Vlatten 1993). Methanol extract also showed great promise as possible stimulants of cockroach trail following behavior and could possibly be used for the directional manipulation of cockroach movements. Unfortunately, the methanol extracts of cockroach fecal material are unsuitable for use indoors because of their unpleasant color and odor.

A new extraction process for German cockroach fecal material was developed that successfully eliminated the color and odor issues associated with traditional methanol extracts. The fecal material was first extracted in methylene chloride and then the filtrate was mixed and separated with water. The final aqueous extract was quite clear and difficult to distinguish from water except by smell. Although the extract had lost the "roachy" odor it still retained and odor similar to that of stale water.

The aqueous extract also retained the attractant activity of the methanol extract described by Rust and Reierson in (1976, 1977). In tests similar to the "bowl assay" (Rust and Reierson 1976) cockroach mortality was significantly enhanced when the aqueous extract was combined with Dursban Pro, (DowAgrosciences, Indianapolis, IN), an emulsifiable concentrate of chlorpyrifos, and Whitmire

PT240, (Whitmire Research Laboratories, Inc., St. Louis, MO) an aerosol formulation of boric acid. The enhanced mortality was due to chemical attractants present in the extract that caused the cockroaches to remain in contact with treated surfaces longer. This allowed the insects to pick up lethal doses of the insecticide.

The methanol extract described by Wendler and Vlatten (1993) and Rust and Reierson (1996, 1997) were used to document trail following behavior in different groups of German cockroaches (adult males, adult females, late instars and gravid females). It was found that for all groups tested the average perpendicular distance from a trail of fecal extract was significantly less than the distance from control trails. However, trail following ability varied among the cockroach groups. Overall, the adult males exhibited superior trail following ability although they were not significantly better than the adult females. Adult female trail following ability was superior to gravid females but was not significantly better than the late instars. Gravid females were shown to have the poorest trail following ability. These results correlated with the differences in foraging frequency observed by Demark in 1992. Demark (1992) determined that the adult male cockroaches foraged more frequently than the other members of the population and that the gravid females foraged the least. This correlation between trail following ability and

foraging frequency indicates that adult males may be more sensitive to directional cues in their environment because they spend more time investigating their surroundings. Gravid females rarely leave the harborage and therefore, have little sensitivity to chemical directional cues.

A relatively low concentration of methanol fecal extract (and subsequently lower concentration of pheromonal chemicals) was required to elicit the trail following response in adult males. The TC_{50} or concentration of fecal extract necessary to stimulate the trail following response in 50% of adult male cockroaches was 5.6% in methanol.

The aqueous extract was also found to elicit trail following behavior in adult male cockroaches. In arena tests the mean percentage of German cockroaches following the trail of aqueous extract was 74%. This was significantly greater than the percentage of cockroaches that followed either of two control trails (22%).

The residual activity of the aqueous extract did not decline over the two week period when the treated trails were stored in air tight refrigerated conditions. However, the activity of trails stored in the open air declined significantly within three days. Trail following activity for trails stored in the open air for one week was only 23%.

Trails of aqueous extract which were positioned between cockroach harborages and sticky traps were found to significantly enhance trap catch. The mean number of German

cockroaches caught in traps supplemented with trails of fecal extract was 28 compared with 11 cockroaches caught in traps with control trails. Although the total number of cockroaches captured was significantly increased by the addition of extract treated trails, the trapping effort was biased toward adult cockroaches. The beginning ratio of adults to nymphs in arena tests was 20:30 (2:3). However, the adult to nymph trap catch in both the treated and control traps was practically 1:1 (1.15:1 and 1.11:1, respectively). The enhanced trap catch and bias toward the capture of adult cockroaches in the arena tests indicated that the trails of fecal extract were more influential on the movement of adult cockroaches than immatures. This observation is substantiated by the superior trail following ability observed for the nongravid adult cockroaches in the trail following bioassays.

Sakuma and Fukami(1990) identified several volatile attractants in German cockroach fecal material. One particular attractant, 1-dimethylamino-2-methyl-2-propanol (DMAMP) was determined to be 50-1000 times more attractive that all others tested. DMAMP was subsequently described as the most significant attractant component in German cockroach aggregation pheromone. Because this component stimulated positive chemo- and anemotaxes in German cockroaches it was evaluated in our trail following bioassay to determine if it had any influence on cockroach trail

following behavior. However, only an insignificant number of the adult male German cockroaches were observed to respond to trails treated with DMAMP in the bioassay.

Previous tests have shown that both the methanol and aqueous extracts of German cockroach fecal material elicited aggregation behavior as well as trail following behavior. However, the amount of aggregation pheromone (DMAMP) in these extracts had never been quantified. The methanol extract was analyzed by gas chromatography in full scan electron impact analysis, selected ion monitoring and chemical ionization modes. DMAMP was not detected in the methanol extract using any of these techniques, and if present, had a concentration in the extract of < 50 ppm.

Based on the observations that trail following behavior is different from aggregation behavior and that DMAMP neither elicited trail following behavior nor was detected in an active fecal extract, I propose that the active component responsible for trail following behavior in the German cockroach is not aggregation pheromone but a different chemical stimulus or chemical combination. I offer to name this chemical(s) "German cockroach trail pheromone."

LIST OF REFERENCES

Alcamo, I. E. and A. M. Frishman. 1980. The microbial flora of field collected cockroaches and other arthropods. J. Environ. Health. 42: 263-266.

Alexander, R. D. 1974. The evolution of social behavior. Annu. Rev. Ecol. Syst. 5: 325-383.

Appel, A. G. 1995. Blattella and related species, pp. 1-19. *In* M. K. Rust, J. M. Owens and D. A. Reierson [eds.], Understanding and controlling the German cockroach. Oxford University Press, New York.

Bell, W. J. 1991. Searching behavior: The behavioral ecology of finding resources. Chapman and Hall, London, England. 358 pp.

Bell, W. J., T. Burk, and G. R. Sams. 1973. Cockroach aggregation pheromone: Directional orientation. Behav. Biol. 9: 251-255.

Bell, W. J., C. Parsons and E. A. Martinko. 1972. Cockroach aggregation pheromones: Analysis of aggregation tendency and species specificity (Orthoptera: Blattidae). J. Kans. Entomol. Soc. 45: 414-421.

Bennett, G. W., E. S. Runstrom and J. Bertholf. 1984. Examining the where and why and how of cockroach control. Pest Control. 52: 42-43, 46, 48, 50.

Bennett, G. W. and C. G. Wright. 1971. Response of German cockroaches to spray constituents. J. Econ. Entomol. 64: 1119-1124.

Benson, E. P. and P. A. Zungoli. 1997. Cockroaches, pp. 122-203. *In* Mallis, A., S. A. Hedges and D. Moreland [eds.], Handbook of pest control, eighth edition. Mallis Handbook and Technical Training Company, Cleveland, Ohio.

Blum, M. S. Pheromonal sociality in the Hymanoptera, pp. 222-249. *In* Birch, M. C. [ed.], Frontiers of biology, Vol. 32, Pheromones. American Elsevier, New York.

Brenner, R. J., K. C. Barnes, R. M. Helm and L. W. Williams. 1991. Modernized society and allergies to arthropods: Risks and challenges to entomologists. Amer. Entomol. 37: 143-155.

Burk, T. and W. J. Bell. 1973. Cockroach aggregation pheromone: Inhibition of locomotion. J. Kans. Entomol. Soc. 46: 36-41.

Butler, D., J. C. Fletcher and D. Walter. 1969. Nest-entrance marking with pheromones by the honeybee, *Apis mellifera* L. and by a wasp, *Vespula vulgaris* L. Animal Behav. 17: 142-147.

Campion, D. G. 1984. Survey of pheromone uses in pest control, pp. 405-449. *In* H. E. Hummel and T. A. Miller [eds.], Techniques in pheromone research. Springer-Verlag, New York.

Cardone, R. V. and J. J. Gauthier. 1979. How long will *Salmonella* bacteria survive in German cockroach intestines? Pest Control. 47: 28-30.

Chapman, M. D., L. D. Vailes, D. E. Mullins, S. M. Squillace, L. E. Gelber, and T. A. E. Platts-Mills. 1992. Cockroach allergens in urban environments are a major risk factor for acute asthma attacks, p. 161. *In*: Robinson, W. H. [ed.]: Proceedings of the National Conference of Urban Entomology. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Cloarec, A., and C. Rivault. 1991. Age-related changes in foraging in the German cockroach (Dictyoptera: Blattellidae). J. Insect Behavior. 4: 661-673.

Cornwell, P. B. 1968. The cockroach, Vol. I Hutchinson, London. 391 pp.

DeMark, J. J. 1992. Behavior of German cockroach, *Blattella germanica* (L.), life stages as measured by computerized moving-image analysis. Ph.D. dissertation, Purdue University, West Lafayette, IN.

Denzer, D. J., M. E. A. Fuchs and G. Stein. 1988. The behavior of *Blattella germanica* (L.): Radius of activity and refuge loyalty. J. Appl. Entomol. 105: 330-343.

Ebeling, W. 1978. Urban entomology. University of California Division of Agricultural Sciences, Berkeley. 695 pp.

Ebeling, W., D. A. Reierson and R. E. Wagner. 1967. Influence of repellency on the efficacy of blatticides. II. Laboratory experiments with German cockroaches. J. Econ. Entomol. 60: 1375-1390.

Ebeling, W., R. E. Wagner and D. A. Reierson. 1966. Influence of repellency on the efficacy of blatticides. I. Learned modification of behavior of the German cockroach. J. Econ. Entomol. 59: 1374-1388.

Fitzgerald, T. D. and J. S. Edgerly. 1979. Specificity of trail markers of forest and Eastern tent caterpillars. J. Chem. Ecol. 5: 565-574.

Fitzgerald, T. D. and E. M. Gallagher. 1976. A chemical trail factor from the silk of Eastern tent caterpillar *Malacosoma americanum* (Lepidoptera: Lasiocampidae). J. Chem. Ecol. 2: 187-193.

Fuchs, M. E. A., S. Franke and W. Francke. 1985. Carbonsauren in kot von *Blattella germanica* (L.) Und inre mogliche rolle als teil des affrefationspheromons. Z. Anf. Ent. 99: 499-503.

Glaser, A. E. 1980. Use of aggregation pheromones in the control of the German cockroach (*Blattella germanica*). Int. Pest Cont. 22: 7-21.

Goldstein, M. and M. Gehret. 1997. Fatal attraction. Pest Cont. Tech. 25: 58-59,62,64,118.

Gupta, A. P., Y. T. Das, J. R. Trout, W. R. Gusciora, D. S. Adams and G. J. Bordash. 1975. Effectiveness of spray-dust-bait combination and the importance of sanitation in the control of German cockroaches in an inner-city area. Pest Control 41: 20-26, 58-62.

Holldobler, B. 1984. Evolution of insect communication, pp. 349-377. *In* Lewis, T. [ed.], Insect communication. 12th Symp. Roy. Entomol. Soc. London. Academic Press, London.

Holldobler, B. and E. O. Wilson. 1990. The ants. Harvard University Press, Cambridge, MA. 732 pp.

Ishii, S. 1970. An aggregation pheromone of the German cockroach, *Blattella germanica* (L.). *In* D. L. Wood, R. M. Silverstein and M. Nakajima [eds.], Control of insect behavior by natural products. Academic Press, New York.

Ishii, S. and Y. Kuwahara. 1967. An aggregation pheromone of the German cockroach *Blattella germanica* L. (Orthoptera: Blattellidae). Appl. Ent. Zool. 2: 203-217.

Ishii, S. and Y. Kuwahara. 1968. Aggregation of German cockroach (*Blattella germanica*) nymphs. Experientia 24: 88-89.

Izutsu, M., S. Ueda and S. Ishii. 1970. Aggregation effects on the growth of the German cockroach, *Blattella germanica* (L.) (Blattaria: Blattellidae). Appl. Ent. Zool. 5: 159-171.

Jettmar, H. M. 1935. Kuchenschaben als krangheitsubertrager. (Cockroaches as vectors of disease.) Wein. Klin. Wechr. 48: 700-704.

Kennedy, J. S. 1978. The concepts of olfactory 'arrestment' and 'attraction'. Physiolog. Entomol. 3: 91-98.

Kitamura, C., H. Koh and S. Ishii. 1974. Possible role of feces for directional orientation of the German cockroach *Blattella germanica* L. (Orthoptera: Blattellidae). Appl. Ent. Zoo. 9: 271-272.

Klotz, J. H. and J. Moss. 1996. Oral toxicity of a boric acid-sucrose water bait to Florida Carpenter ants (Hymenoptera: Formicidae). J. Entomol. Sci. 31: 9-12.

Klotz, J. H. and B. L. Reid. 1992. The use of spatial cues for structural guideline orientation in *Tapinoma sessile* and *Campanotus pennsylvanicus* (Hymneoptera: Formicidae). J. Insect Behav. 5: 71-82.

Koehler, P. G., H. R. Agee, N. C. Leppla and R. S. Patterson. 1987. Spectral sensitivity and behavioral response to light quality in the German cockroach (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. 80: 820-822.

Koehler, P. G. and R. S. Patterson. 1986. A comparison of insecticide susceptibility in seven nonresistant strains of the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae). J. Econ. Entomol. 23: 298-299.

Koehler, P. G., R. S. Patterson and R. J. Brenner. 1990. Cockroaches, pp. 101-174. *In*: A. Mallis: Handbook of pest control. Franzak and Foster Co., Cleveland, Ohio.

Koehler, P. G., C. A. Strong and R. S. Patterson. 1994. Rearing improvements for the German cockroach (Dictyoptera: Blattellidae). J. Med. Entomol. 82: 163-170.

Kopanic, R. J., C. Schal. 1997. Relative significance of direct ingestion and adult-mediated translocation of bait to German cockroach (Dictyoptera: Blattellidae) nymphs. J. Econ. Entomol. 90: 1073-1079.

Mackerras, M. J. and I. M. Mackerras. 1948. An epidemic of infantile gastroenteritis in Queensland caused by *Salmonella bovis-morbificans* (Basenau). J. Hygiene 47: 166-181.

Maschwitz, U. 1975. Old and new trends in the investigation of chemical recruitment in ants, pp. 47-59. *In* Noirot, C., P. E. Howse and G. Le Masne, [eds.], Pheromones and defensive secretions in social insects. French Section of IUSSI, Proceedings, University of Dijon.

McFarlane, J. E. 1984. Repellent effect of volatile fatty acids of frass on larvae of German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). J. Chem. Ecol. 12: 1617-1622.

McFarlane, J. E. and I. Alli. 1986. Aggregation of larvae of *Blattella germanica* (L.) by lactic acid present in excreta. J. Chem. Ecol. 12: 1369-1375.

Miller, D. M., P. G. Koehler, and R. S. Patterson. 1996. Influence of German cockroach (Dictyoptera: Blattellidae) fecal extract on food consumption and harborage choice. J. Econ. Entomol. 89: 668-672.

Miller, D. M., P. G. Koehler, and R. S. Patterson. 1997. Use of German cockroach (Dictyoptera: Blattellidae) fecal extract to enhance toxic bait performance in the presence of alternative food sources. J. Econ. Entomol. 90: 483-487.

Moore, W. S. and T. A. Granovsky. 1983. Laboratory comparisons of sticky traps to detect and control five species of cockroaches. J. Econ. Entomol. 76: 845-849.

Mori, K. 1996. Recent results in pheromone synthesis. Pure and Appl. Chem. 68: 2111-2118.

Morrell, C. C. 1911. The bacteriology of the cockroach. Br. Med. J. 2: 1531-1532.

Ogg, C. L. 1989. Factors influencing surface preference by the German cockroach (*Blattella germanica* L.). Thesis-MS University of Nebraska, Lincoln.

Owens, J. M. 1995. Detection and monitoring. *In* M. K. Rust, J. M. Owens and D. A. Reierson [eds.], Understanding and controlling the German cockroach. Oxford University Press, New York.

Owens, J. M. and G. W. Bennett. 1983. Comparitive study of German cockroach population sampling techniques. Env. Entomol. 12: 1040-1046.

Peterson, S. C. and T. D. Fitzgerald. 1991. Chemoorientation of Eastern tent caterpillar to trail pheromone, 5-beta-cholestane-3,24-dione. J. Chem. Ecol. 17: 1963-1972.

Pio, C. A., and R. M. Harrison. 1987. Vapour pressure of ammonium chloride aerosol: Effect of temperature and humidity. Atmos. Environ. 21: 2711-2715.

Pulliam, H. R. 1973. On the advantages of flocking. J. Theor. Biol. 38: 419-422.

Ritter, F. J. and C. J. Persoons. 1975. Recent developments in insect pheromone research, in particular in the Netherlands. Neth. J. Zool. 25: 261-275.

Ross, M. H., B. L. Bret and C. B. Keil. 1984. Population growth and behavior of *Blattella germanica* (L.) (Orthoptera: Blattellidae) in experimentally established shipboard infestations. Ann. Entomol. Soc. Am. 77: 740-752.

Ross, M. H. and K. R. Tignor. 1986. Response of german cockroaches to aggregation pheromone emitted by adult females. Entomol. Exp. Appl. 41: 25-31.

Rust, M. K., T. Burk, and W. J. Bell. 1975. Pheromone-stimulated locomotory and orientation responses in the American cockroach. Anim. Behav. 24: 52-67.

Rust, M. K., J. M. Owens, and D. A. Reierson. 1996. Understanding and controlling the German cockroach. Oxford University Press, New York. 430 pp.

Rust, M. K. and D. A. Reierson. 1976. Using pheromone extract to reduce repellency of blatticides. J. Econ. Entomol. 70: 34-38.

Rust, M. K. and D. A. Reierson. 1977. Increasing blatticidal efficacy with aggregation pheromone. J. Econ. Entomol. 70: 693-696.

Sakuma, M. and H. Fukami. 1985. The linear olfactometer: An assay device for taxes of the German cockroach, *Blattella*

germanica (L.) (Dictyoptera: Blattellidae) toward their aggregation pheromone. Appl. Ent. Zool. 4: 387-402.

Sakuma, M. and H. Fukami. 1990a. Dose/respone relations in taxes of nymphs of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) to their aggregation pheromone. Appl. Ent. Zool. 25: 9-16.

Sakuma, M. and H. Fukami. 1990b. The aggregation pheromone of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae): Isolation and identification of the attractant components of the pheromone. Appl. Ent. Zool. 25: 355-368.

Sakuma, M. and H. Fukami. 1993a. Novel steroid glycosides as aggregation pheromone of the German cockroach. Tetrahedron Lett. 34: 6059-6062.

Sakuma, M. and H. Fukami. 1993b. Aggregation arrestant pheromone of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae): Isolation and structure elucidation of blattellastanoside-A and -B. J. Chem. Ecol. 19: 2521-2541.

Sakuma, M., H. Fukami and Y. Kuwahara. 1996a. Aggregation pheromone of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae): Controlled release of attractant amines by salt formation. Appl. Entomol. Zool. 32: 143-152.

Sakuma, M., H. Fukami and Y. Kuwahara. 1996b. Attractiveness of alkylamines and aminoalcohols related to the aggregation attractant pheromone of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). Appl. Entomol. Zool. 32: 197-205.

SAS Institute. 1988. SAS/STAT Guide for personal computers. SAS Institute, Cary, NC.

Schulaner, F. A. 1970. Sensitivity to cockroaches in three groups of allergic children. Pediatrics. 45: 465.

Silverstein, T. M. 1981. Pheromones: Background and potential for use in insect pest control. Science. 213: 1326-1331.

Stejskal, V. 1997. Distribution of faeces of the German cockroach, *Blattella germanica*, in a new refuge. Entomol. Exp. Appl. 84: 201-205.

Strek, M., R. V. Peterson, and R. L. Alexander. 1979. Retention of bacteria in the alimentary tract of the

cockroach, *Blattella germanica*. J. Environ. Health. 41: 212-212.

Strong, C. A. , P. G. Koehler and R. S. Patterson. 1993. Oral toxicity and repellency of borates to German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 87: 389-397.

Tobin, T. R. 1981. Pheromone orientation: Role of internal control mechanisms. Science 214: 1147-1149.

Wendler, G. and R. Vlatten. 1993. The influence of aggregation pheromone on walking behavior of cockroach males (*Blattella germanica* L.) J. Insect Physiol. 39: 1041-1050.

Wileyto, E. P., G. M. Boush and L. M. Gawin. 1984. Function of the cockroach (Orthoptera: Blattidae) aggregation pheromone. Environl Entomol. 13: 1557-1560.

Wilson, E. O. 1961. Chemical communication among workers of the Fire ant, *Solenopsis saevissima* (Fr. Smith) 3. The experimental induction of social responses. Anim. Behav. 10: 159-164.

BIOGRAPHICAL SKETCH

Dini Michele Miller was born in La Mesa, California, December 17, 1962, to Diana F. Miller and Micheal R. Miller. She grew up in the San Diego area and graduated from Helix High School in 1981. While working for a local department store she began attending night school at Grossmont Community College. Dini transferred to the University of California at Los Angeles in the fall of 1988. She graduated from UCLA magna cum laude, receiving her B.A. in geography/ecosystems in 1991. She joined the Department of Entomology and Nematology at the University of Florida in 1992 working under Dr. P. G. Koehler. As a master's student in urban entomology, Dini conducted research on the potential contribution of German cockroach aggregation pheromone to urban pest management programs. In 1994 Dini began her doctoral program under Dr. Koehler. Her research focused primarily on the documentation of German cockroach trail following behavior. Dini Miller's research and scholarship was recognized by the Entomological Society of America in 1998 with the presentation of the John Henry Comstock Memorial award. Dini is engaged to be married on Dec. 20, 1998 to Mr. Timothy McCoy of Hollywood, Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Philip G. Koehler, Chair
Professor of Entomology
and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James L. Nation
Professor of Entomology
and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard J. Brenner
Assistant Professor of
Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Olaf N. Nesheim
Professor of Food Science
and Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1998

Dean, College of
Agriculture

Dean, Graduate School